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LEAD

ENVIRONMENTAL SOURCES AND RED CELL TOXICITY IN URBAN CHILDREN

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PREPARED FOR
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JUNE 1975

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LEAD:
ENVIRONMENTAL SOURCES AND RED CELL TOXICITY IN URBAN CHILDREN

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16. ABSTRACT

A comprehensive environmental study was carried out for correlation of lead in multiple sources with the increased blood lead of urban children. In the three age groups, 2-5, 10-12, and 14-18 years, urban children had higher blood leads than their suburban counterparts, although the difference decreased with age. The increased blood lead correlated with increased lead in the urban dustfall, yard soil and boot tray lead. There was no significant urban-suburban difference in air lead, housedust lead, available paint chips or lead in milk and water. Lead in yard dirt and blood lead both correlated with residential proximity to traffic.

Although all blood lead were below 40 µg/dl, there was a significant linear decrease in red cell (rbc) membrane Na/K ATPase as blood lead increased; children with a blood lead above 20 µg/dl had decreased activity of rbc glutathione and increased rbc G-6-PD and 6-PGD. The increase in the latter two enzymes supports decreased rbc survival at the level of lead exposure of urban children. Iron deficiency anemia showed no correlation with blood lead and the administration of iron supplements to preschool children resulted in higher blood leads than their matched controls.

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ENVIRONMENTAL SOURCES AND RED CELL TOXICITY IN URBAN CHILDREN

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ABSTRACT

A comprehensive environmental study in Omaha for the correlation of lead from multiple sources with the blood lead of children shows a significant decrease in air and dustfall lead between 1970 and 1974. Despite this, urban children have higher blood leads than their suburban counterparts in all three age groups, 2-5, 10-12, and 14-18 years. The higher blood lead correlates with the urban dustfall, yard soil and boot tray dirt and, in the preschool ages, with lead in interior dust. Soil lead and blood lead both correlate with residential proximity to traffic. There was no significant urban-suburban difference in lead in the air, milk, or available paint chips.

There is a significant linear decrease in red cell (rbc) membrane Na/K ATPase as blood lead increases from 10 to 80 µg/dl. In association with inhibition of this transport enzyme there is shortened rbc survival manifest in increased rbc G-6-PD and 6-PGD. There is selective inhibition of rbc glutathione at a blood lead above 20 µg/dl. Iron deficiency does not correlate with blood lead and the administration of iron supplements to preschool children does not decrease their blood lead.

This report was submitted in fulfillment of Grant Number R 802043 by the University of Nebraska College of Medicine, under the sponsorship of the Environmental Protection Agency. Work was completed as of October 31, 1974.

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Mrs. Marilyn Schlicht served as project coordinator and directed all of the school sampling teams. Mrs. Kathryn Stelmak, M.S., Chief of the Laboratory, was ably assisted by Mr. Warren Hill.

SECTION I

CONCLUSIONS

Lead in the air and dustfall in Omaha markedly declined from 1970 to 1974, coincident with the antipollution measures enacted by the EPA. Lead in both urban and suburban air fell to neglible amounts in 1973, the time of introduction of unleaded gasoline. Studies subsequent to the time of this report show a concommitant and significant drop in the blood lead of urban school children from 1971 to 1973 to 1975.

The decrease represents a prompt and gratifying response to the Clean Air Act but stimulates an even closer scrutiny of the threshold for health effects and the investigation of previously unrecognized toxicity to lead.

Despite the decreases in environmental lead, the blood lead of urban children and adolescents continues to be higher than that of their suburban counterparts in all three age groups: 2-5, 10-12, and 14-18 years. The urban-suburban difference is highest in preschool children and decreases with age: the younger the child, the greater the exposure.

The increase in blood lead in urban children of school age correlates with the increased lead in urban dustfall, yard soil and dirt brought into the house. Lead in yard dirt and blood lead correlate with residential proximity to traffic and industry. In preschool urban children, ages 2-5, blood lead also correlates with the lead concentration in interior dust as well as with lead in playground soil. In this study, correlation of blood lead with dustfall and dirt is much more evident than with the lead in air, milk, water, or in available paint chips.

General environmental exposure relative to age appears to be a more significant factor in the elevation of the blood lead of urban children than intrinsic differences in red cell metabolism. Blood lead is not increased in children with iron deficiency. Although urban black children have both a higher lead and lower hemoglobin than suburban white children, within any age group of environmentally homogenous children, blood lead increases with the serum iron. The administration of ferrous sulfate, 200 mg/day, to preschool children with iron

deficiency does not decrease the blood lead. To the contrary, data from this study suggests that an increase in iron intake may actually increase the blood lead. This might relate to the effect of ferritin binding of lead or to facilitation of a common mucosal transport mechanism for heavy metals.

Lead toxicity is not an "all or none" phenomenon and the consideration of a blood lead of 40 µg/dl as the threshold for adverse effects denies evidence for an actual continuum of toxicity. This continuum is shown by the fact that children with blood leads increased to 20-40 µg/dl have significant depression of red cell membrane Na/K ATPase, the enzyme considered critical to membrane stability, cation transport, and cell size. They have evidence of a shortened red cell survival as reflected in increased G-6-PD, enzymes that indicate a young red cell population. They also have a decrease in red cell glutathione. Glutathion, an index of red cell resistance to oxidation, normally increases in a young red cell population, and its decrease in young cells suggests a selective inhibition by lead.

This study demonstrates that an adverse health effect, decreased red cell survival, occurs at "non-toxic" blood lead levels found even at the reduced environmental exposure attributed to the successful antipollution measures of the EPA.

SECTION II

RECOMMENDATIONS

We have evidence that the urban child is exposed to significantly greater amounts of lead from general environmental sources than is the suburban child. Since this "sub-toxic" increase in blood lead of urban children is associated with the adverse health effect of decreased red cell survival, we strongly recommend continuing efforts to decrease the high fallout of lead particles in the urban dustfall, soil, and house dust.

We recommend investigation of the health effects of an inhibition of red cell membrane Na/K ATPase by a standard protocol in several areas of the United States to demonstrate its significance in ethnic groups of differing environmental exposure and intrinsic differences in red cell metabolism.

Decrease in the Na/K ATPase activity of the red cell membrane at blood lead levels as low as 20 µg/dl also leads us to recommend the study of the effect of lead on Na/K ATPase activity in leucocytes and platelets. We recommend a study of leucocytic membrane transport as a factor in the altered immune response of lead exposure. Since a decrease in platelet Na/K ATPase is associated with a decreased uptake of serotonin, and may parallel a similar decrease in cerebral uptake of serotonin[1], we recommend investigation of the effect of lead on the platelet enzyme. This could provide an available peripheral index of neurotoxicity from lead.

A blood lead threshold for children of 40 µg/dl is no longer physiologically tenable. We propose that the Environmental Protection Agency review and evaluate all current physiologic and biochemical barometers of adverse health effects and make recommendations regarding the standards for acceptable levels of environmental lead exposure.

The demonstrated success of the EPA in reducing environmental lead and investigating its threshold effects is a model that should be applied to other toxic metals such as arsenic, cadmium, and mercury.

SECTION III

INTRODUCTION

There is much evidence that the urban population has a higher blood lead and a higher total body burden of lead than the rural or even the suburban population[2]. In any population, children are considered particularly at risk for an increase in the body burden of lead and its health effects, a fact attributed to the higher load per unit of weight, the higher metabolic rate, and the smaller reservoir of cancellous bone for lead storage[3,4]. There is evidence that the current level of lead in the general urban environment accounts for an increased blood lead in urban children, and may result in low level toxicity[2,3]. This study focuses on three questions basic to the lead exposure of urban children:

A. Environmental sources
B. Intrinsic differences in metabolism
C. Health effects.

A. ENVIRONMENTAL SOURCES

The report is first concerned with definition of the role of general environmental exposure as distinct from unique factors such as deteriorated housing, food contamination and pica. Definition of the contribution of the general sources of lead - air, dustfall, soil, water and milk - is basic to the projection of the effects of a continued increase in environmental lead.

Lead in urban air is a major concern since the absorption of inhaled lead ranges from 30% to 50%[3]. Under controlled conditions, the elevation of air lead above 2 $\mu g/M^3$ produces a discernable increase in blood lead[5]. Our study investigates urban-suburban differences in air lead as related to blood lead, and includes an assessment of seasonal changes in air lead as related to seasonal changes in blood lead.

he contribution of particulate fallout of air lead to the total ingested lead is important. Although the usual intestinal absorption of lead is only 10%, Delves has evidence that it may be as high as 50% in young children[6]. Since an oral lead intake of only 0.6 - 1.0 mg/d causes an increased blood lead and cumulative increases in body burden[3,4], the current levels of general urban exposure are significant. Street dirt, dustfall, yard dirt, milk, water, and food are all general environmental sources of lead with concentrations that often exceed 0.5 mg/gm[7,8]. Interior sources of lead, even in the absence of flaking paint and plaster of older housing, are reflected in the lead content of household dust. Lead in house dust may exceed 5 mg/gm and significantly contribute to the body burden of lead in children without pica[9]. Within any environmentally homogenous population, individual variations in the lead load may result from increased mouthing behavior plus exposure to sources that may be unique to the individual or to the household such as water of low pH, cooking utensils, leaded pottery, lead painted pencils and toys, food selection, ink and paper ingestion, and even toothpaste[10,11].

B. INTRINSIC METABOLIC DIFFERENCES

Within culturally similar populations of the same age group, there also are variations in the blood lead and in the susceptibility to lead toxicity that support intrinsic differences in the absorption or metabolism of lead. Black children in New York City, for example, have higher blood leads than do Puerto Rican children living in apparently identical circumstances[12]. Children with sickle cell disease seem particularly susceptible to lead neuropathy[13,14]. We previously investigated the contribution of a deficiency of red cell G-6-PD, a common metabolic phenomenon found in 10-12% of black males. We did find some suggestion that red cell lead, but not whole blood lead, was increased in children with this genetic difference in molecular chemistry[15]. This suggested the possibility that other disorders of red cell metabolism common in the urban population, such as iron deficiency anemia and sickle cell trait, might alter red cell binding and, by virtue of the increased concentration gradient, enhance the rate of transport of lead from red cell to tissue[16].

Iron deficiency, the most common nutritional deficiency of urban children, has a provocative epidemiologic association with lead poisoning. Six and Goyer[17] showed marked enhancement of tissue lead in iron deficient rats. Ferritin and transferrin have both been shown to bind lead and this binding appears to relate to the increased tissue lead of iron deficient animals[18,19]. The second focus of the study is the evaluation, within a homogenous population, of the correlation of serum iron and blood lead. It includes a clinical trial of the effect of oral iron supplements on the blood lead of iron deficient children to test the experimental evidence that iron supplementation may prevent lead toxicity.

C. HEALTH EFFECTS

The third area of concern is the threshold for health effects of the increased body burden of lead resulting from current urban levels of exposure. Of all the indices of body burden available, blood lead correlates best with tissue lead, although not with bone lead, and is used throughout as evidence of lead load. Multiple clinical and biochemical correlations, as reviewed by de Bruin[20], support the concept that lead toxicity is a continuum and not an all-or-none phenomenon occurring at a specific blood level such as 40 or 80 µg/dl.

The effects of lead on the red cell survival have long been known and are well reviewed by Hernberg[21], but have been less used than the suppression of erythropoiesis as clinical and biologic evidence of lead toxicity. In vivo and in vitro, lead exposure results in rapid loss of potassium from the red cell, leaving a shrunken cell with a decreased capacity for survival.

Although changes in osmotic resistance and decreased red cell survival are well documented in acute lead poisoning[22-25], the complexities of measurement of red cell survival have impeded broad scale clinical studies at low levels of blood lead. Red cell enzyme activity is a reliable indirect measurement of cell age[26]. Hexokinase, aldolase, GOT, G-6-PD, 6-PGD are all significantly higher in young red cells than old[27,28]. A young red cell population is evidence of decreased survival; its correlation with minimal increases of blood lead is evidence of a health effect at levels far below 40 µg/dl.

The integrity of the red cell membrane relates to the cationic transport system of membrane Na/K ATPase[29,30]. Red cell membrane Na/K ATPase has been found to be depressed in lead workers and even in urban adults with blood leads above 30 µg/dl[31-33]. Although some aspects of the experimental suppression of K transport into the red cell are all or none, as discussed by Passow[34], the usual effect of enzyme inhibition is linear. Linear inhibition of red cell membrane Na/K ATPase at subtoxic levels of lead would be a significant health effect and would also support the significance of similar effects of lead on cation transport across cellular and mitochondrial membranes of tissue other than the red cell, and as shown by Hexum in neuronal mitochrondria[35], and suggested by Goyer in renal tubular cells[36].

SECTION IV

DESIGN

LOCATION

The entire study was carried out in Omaha, Nebraska, a city of 350,000 population. Omaha's air has long been known to have a high particulate concentration[37]. Back in 1967, dustfall analysis by the National Air Pollution Control Network had shown downtown Omaha to have the highest dustfall lead of 22 midwestern cities[38]. Since traffic density in Omaha should be commensurate with the low population density, it is assumed that the high air lead and dustfall lead in central Omaha relate to industrial sources. Figure 1 is an outline map of Omaha, showing the urban and suburban areas of study.

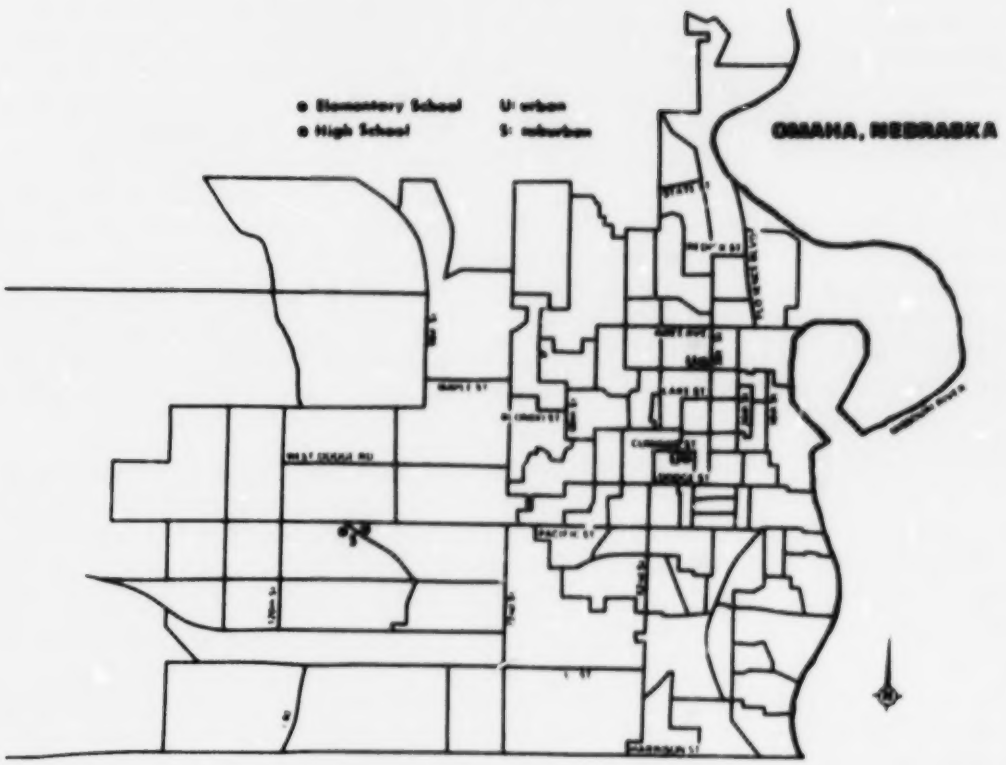

Figure 1. Map of Omaha reproduced at a scale of ⟷ = 1.5 miles. The west suburban area is a 3 mile radius centering on the two suburban schools (S). The urban area is in the northeast quadrant and is a rectangular area bordered by Ames to the north, Dodge to the south, 16th Street on the east, and 42nd Street on the west. The urban grade school (U, solid dot) has a battery manufacturer as its immediate north neighbor. Air and dustfall were sampled at the two urban sites and at the common suburban site, 1971-1974.

Studies of blood lead (Pb-B) in school children as related to environmental lead were conducted in two general areas, 1) a suburban area in southwest Omaha predominantly white and middle class in population where studies were conducted at 1 high school, 1 grade school and 2 day care centers, 2) a northcentral urban industrial area with a predominantly black population with studies conducted at 1 high school, 1 junior high, 9 grade schools and 5 day care centers.

The northcentral area (Figure 2) has a significant amount of substandard housing and has 3 known lead emission sources. There is a small battery manufacturer in the north, immediately adjacent to a grade school. Immediately southwest of the area there is a major lead smelter and a battery reclamation plant. The windrose data for Omaha (Figure 3) shows the worst residential area to be downwind from both lead emission areas.

School children, ages 6-12, living in this high lead area were studied in 1970-71 for the interaction of G-6-PD deficiency and Pb-B[15]. Topographic analysis of the Pb-B of each of 107 school children studied in 1970 in the northcentral area is shown in Figure 4[40]. This shows a sharp peak in the blood lead of children living in the proximity of a small battery paint. A significantly increased Pb-B was also demonstrated in those children living within 500 feet of a major trafficway. The census tract data on housing in the area was analysed but the blood lead of these children, ages 6-12 years, did not show a significant correlation with the percent of deteriorated housing within the census tract. The same geographic areas are used in our present study for the correlation of environmental lead with the Pb-B in high school students 14-18, grade school students, 10-12, and preschool children, 2-5 years old.

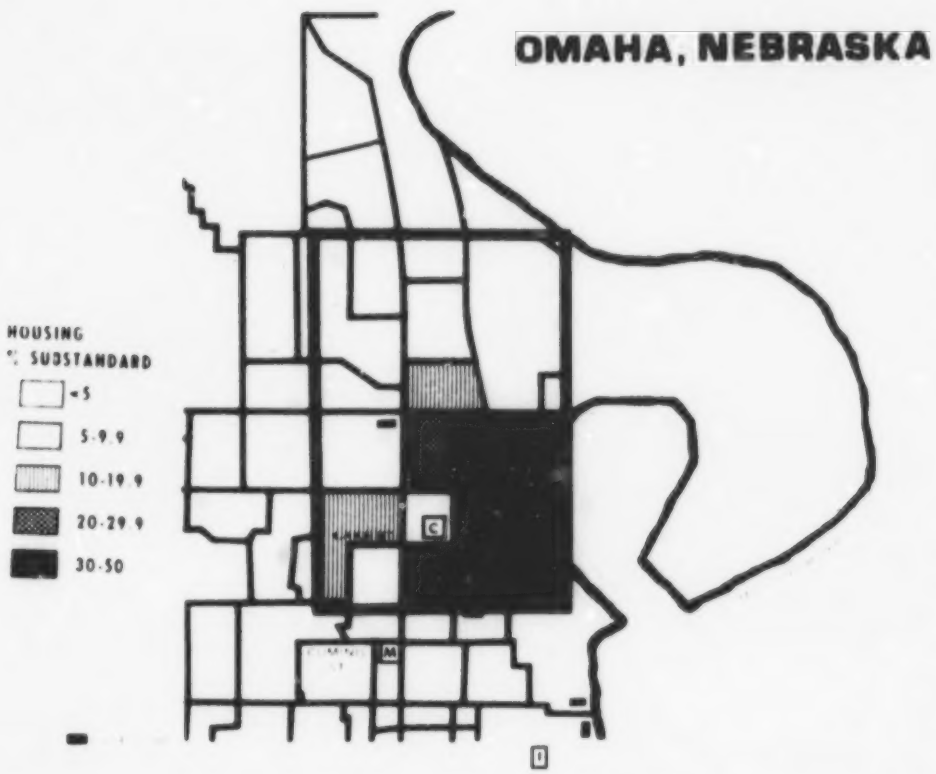

Figure 2: Close up of the northcentral area of Figure 1 showing the percent of substandard housing according to census tracts[39] and the location of 3 lead emission sources. Air sampling was conducted at 3 sites: M (mixed commercial) at the urban high school, 1970-1974; C (commercial) in 1970; and at the urban grade school just south of the battery plant in the center of the area, 1971-1974.

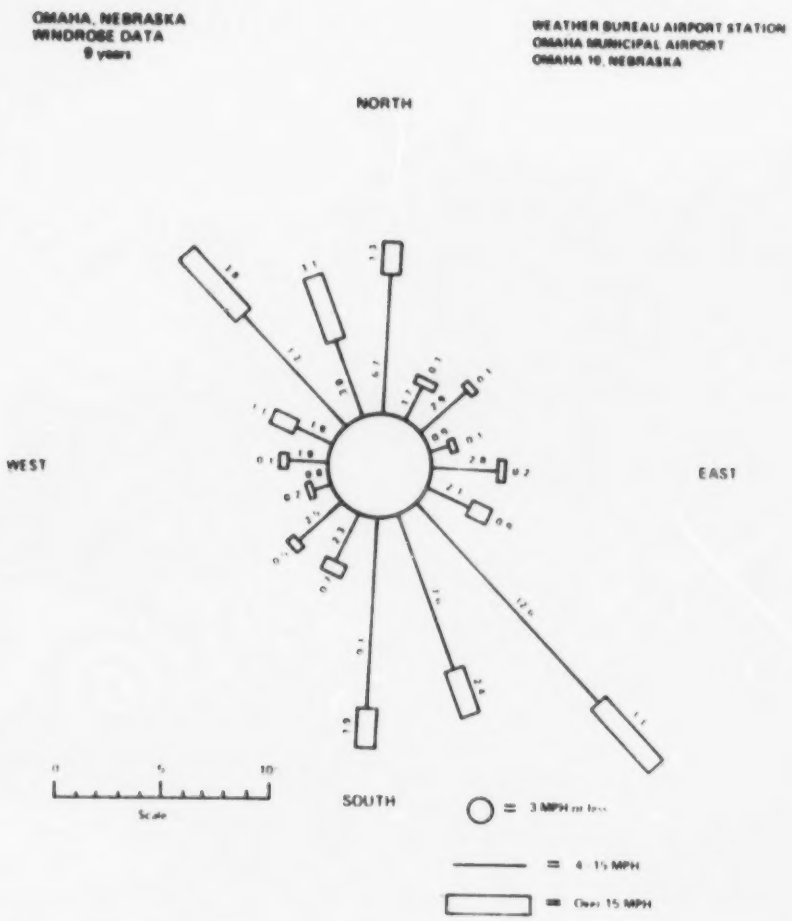

Figure 3: Wind rose data from Omaha showing the prevailing NW and SE winds which tend to focus emissions from the lead sources on the north central urban area of Figure 2.

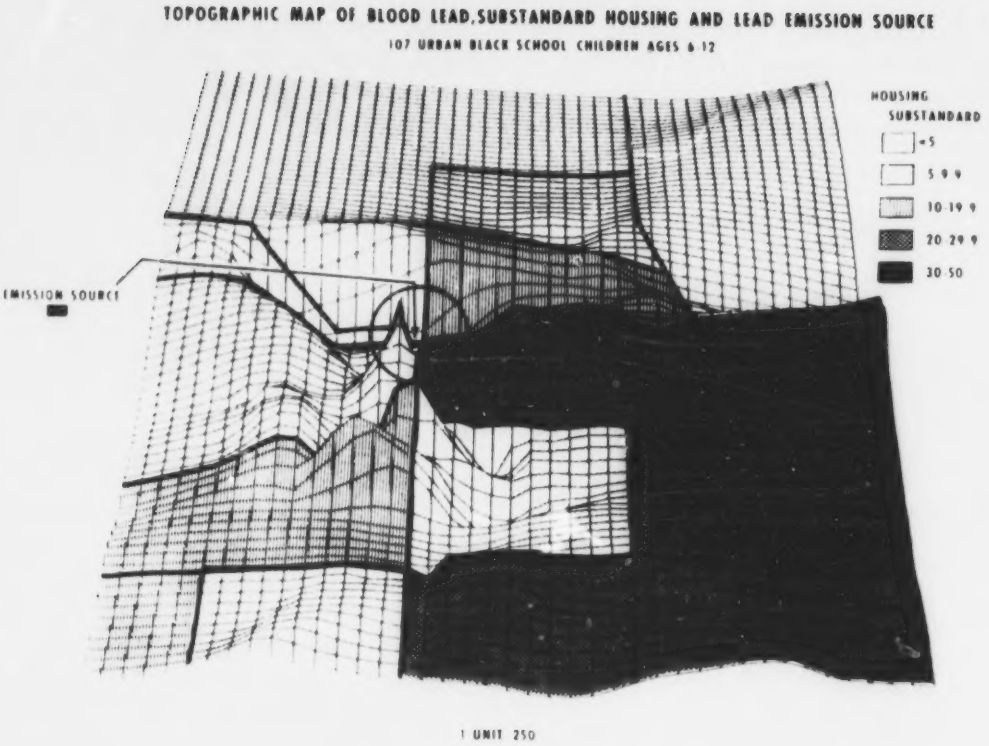

Figure 4: Topographic plot of the blood lead of 107 children living in the northcentral area outlined in Figure 2. The thickest black lines define streets carrying more than 3500 vehicles per lane per day. There is a sharp peak of blood leads (Pb-B) of children living near the central battery plant and a ridge of increased values parallelling the major north-south street. Little correlation of Pb-B with housing is seen[40].

Extremely important to this environmental assessment are the milk and water supplies. In the Omaha area all the milk sold derives from a common pool and is subsequently bottled at individual plants. All water is from the 2 municipal processing plants, both yielding water at pH 8.0 - 9.0 and therefore unlikely to leach lead from pipes in the absence of a water softener. The same grocery chains supply both urban and suburban Omaha, and although general food supplies are similar, variances in selection are assumed.

A. ENVIRONMENTAL STUDIES

The design of the environmental studies was based on the correlation of the following measurements with the blood lead for group and individual analysis. The specific details concerning dates, numbers of samples, and numbers of subjects are given in Section VI.

Air Lead:	2 urban, 1 suburban site, 1970-1974, for group correlation.
Dustfall Lead:	2 urban, 1 suburban site, 1973-1974, for group correlation.
Soil Lead:	7 urban and 4 suburban schools for group analysis. Homes of 14 urban and 14 suburban students for correlation with individual Pb-B and with residential distance from traffic.
Interior Dust:	7 preschools for group analysis.
Paint Chips:	As available.
Water:	10 urban and 10 suburban homes for group and individual correlation.
Milk:	10 suburban and 10 urban homes for group and individual correlation.

The correlations of the environmental lead were as follows:

1. Urban vs suburban, ages 2-5, 10-12, 14-18 years.

2. Group environmental studies for correlation of lead in air, dustfall, milk, and water vs Pb-B of:

    a) urban high schools

    b) suburban high schools

    c) urban grade schools

    d) suburban grade school.

3. Exterior and interior dirt at day care centers vs mean Pb-b at each center.

4. Individual correlations of Pb-B with lead in yard dirt, boot tray dirt, housedust, residential distance from traffic.

B. INTRINSIC METABOLIC DIFFERENCES

Iron Deficiency

The studies on iron deficiency involved screening in January, 1974 of 154 preschool children at 5 urban and 2 suburban day care centers for comparison of Pb-B, serum iron (Fe), iron binding capacity (TIBC), transferrin saturation (% Fe Sat), complete blood count (CBC), red cell indices, and 2,3 DPG. Within each group, correlation was made of blood lead with serum iron and hemoglobin.

After the January screening, 3 groups were chosen for the response to oral ferrous sulfate or placebo. All subjects had a low serum Fe (below 80 µg/dl) but no anemia (Hgb > 10.5 gm%). The general design of the study is diagrammed in Figure 5:

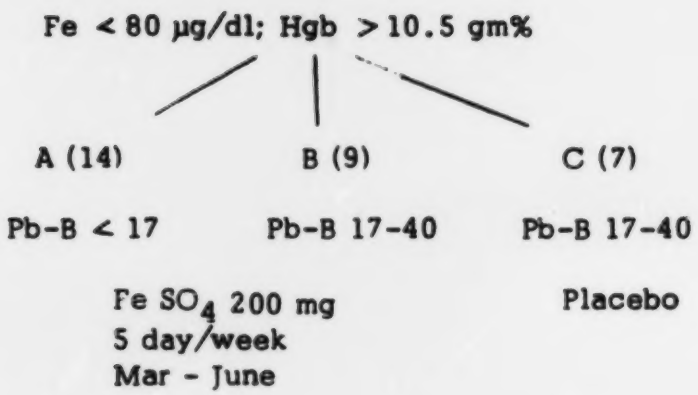

Figure 5: Iron Therapy Study: The response of serum Fe and Pb-B to oral iron was studied in 3 groups; A: 14 low lead children receiving ferrous sulfate, B: 9 high lead children receiving ferrous sulfate, C: 7 high lead children receiving a placebo. Groups B and C were paired for comparable levels of blood lead and serum iron and a double blind system was used for administration of iron.

C. HEALTH EFFECTS

Total and Na/K ATPase were correlated with blood lead in 5 groups:

1. Ages 2-5 years
2. Ages 10-18 years
3. G-6-PD deficient, ages 10-18
4. Normal adults
5. Lead workers.

Evidence of hemolysis or other effect of lead was obtained by correlation of G-6-PD, 6-PGD, 2,3 DPG, GSH with blood lead in the above 5 groups.

SECTION V

OBJECTIVES

1. Definition of the contribution of general environmental sources of lead: air, dustfall, dirt, milk, and water to the higher Pb-B of urban children and assessment of the relative significance of general environmental increases of lead and of sources unique to a household.

2. Investigation of the role of intrinsic differences in metabolism on Pb-B: the effects of iron deficiency and the possible beneficial effect of oral iron supplements to correct the iron deficiency.

3. Investigation of the hemolytic effect of lead, even at Pb-B levels below 40 μg/dl, as manifested by red cell membrane Na/K ATPase and by enzymes indicating decreased red cell age.

SECTION VI

DEVELOPMENT OF QUANTITATIVE METHODS

MATERIALS AND METHODS

A. ENVIRONMENTAL STUDIES

The urban and suburban areas of this study are shown in Figure 1. Students live and attend school within their urban or suburban area. At ages 1-12, urban children attend school within 0.25 miles of home; suburban children live within a 5 mile radius. All high school students studied live within a 3 mile radius of their respective schools, but greater mobility is assumed in the high school age group. Comparison is made of the blood lead and environmental lead of 4 groups:

1. Urban vs suburban high school students, ages 14-18 (1973).

2. Urban children, ages 10-12, vs suburban children, ages 10-12 (1973).

3. Urban children, ages 10-12 years, with blood lead over 20 µg/dl vs those with blood lead below 20 µg/dl (1973).

4. Urban children, ages 2-5, attending 5 day care centers vs preschool children at suburban centers (1974).

Blood Lead

Blood lead (Pb-B) studies in the 4 paired populations were as follows:

1. High School, Urban vs Suburban -

Venous samples in February and April, 1973 for micro Pb-B by the Delves micro method[41], and macro Pb-B by the Farrelly & Pybus method[42], plus 3 fingerstick samples for micro lead in January, March, and May, 1973. The lead content of fingerstick samples were corrected for the ratio of venous to capillary hemoglobin.

2. Grade School, Urban vs Suburban -

Fingerstick samples for micro assay in 35 urban black children in December, 1972 and in 35 suburban children in early January, 1973.

3. Urban Grade School, High Lead vs Low Lead -

Of the 35 urban black children, 26 had a second fingerstick lead in February, 1973, and a venous sample in April, 1973, for both a micro and a macro assay of Pb-B. Fingerstick Pb-B leads of December and February were corrected to the venous hemoglobin of April.

4. Preschool Children -

154 venous samples for micro Pb-B were collected from 92 urban children, predominately black, and 31 suburban children, predominately white, all ages 2-5. Fifty of the urban children and all of the suburban children were sampled in January, 1974, and 45 children were sampled in April, 1974. Repeat Pb-B were obtained in July, 1974 from 30 of the children.

Environmental Lead

Air lead was collected as random 24-hour Hi Vol Samples at 3 sites: 1) the urban high school at 15' elevation, 2) the urban grade school (located next to a small battery plant) with samples at both 3' and 15', 3) the suburban high school at 15'. The monthly values are the mean of 6 to 20 24-hour samples per month collected during 7 months of 1970, 8 months of 1972-1973, and 10 months of 1974.

Dustfall lead was collected in plastic buckets in 30 day samples at the same 3 sites as the air lead for 6 months of 1973 and 4 months of 1974.

Soil lead was collected at the 2 high schools, the 2 grade schools, the 7 preschool centers and at 37 homes (7 urban high school, 7 suburban high school, 16 urban grade school and 7 suburban grade school). At each site, 4 samples were obtained, each consisting of a 2" core collected at a distance halfway between the building and the lot margin on all 4 sides of the building.

Interior dust was sampled in two ways: dust as emptied from the vacuum cleaner or, if no vacuum cleaner was in the home, as a floor sweeping. Dirt brought into the house was collected over 30 days in boot trays fitted with a plastic grid and placed in the entry way.

Paint chips were sampled as availabe from flaking interior or exterior paint.

Air, dustfall, and soil lead were measured by AA spectrometry after nitric acid extraction by E.R. Williams, D.P.H., South Carolina Department of Health and Environmental Control in 1973. Additional dustfall samples from 1973 and all environmental samples from 1974 were similarly assayed by the Omaha-Douglas County Health Department.

Water was collected at 20 homes in 1973 as one month random collections. Each householder was given a 1 liter plastic jug and asked to add approximately 15 ml every morning (before running water for other purposes) and 15 ml every evening after dinner. Water was analysed by the Delves cup assay after nitric acid extraction (20 samples) by Dr. Williams.

All of the study homes used whole milk only. At the time of the soil sampling, purchase was made of the same brand of whole milk as used by each household. Lead assay was by the Delves cup assay (20 samples) of Haelen, Cooper and Pampel[43] in our laboratory.

B. LEAD AND IRON DEFICIENCY

Initial screening of venous blood for blood lead (Pb-B) by the Delves micro method[41], serum iron (Fe) and total iron binding capacity (TIBC), 2,3 DPG[44], CBC and hematologic indices was carried out in January, 1974, on 154 preschool children, ages 2-5 years. Of these, 31 white children attended 2 suburban day care centers and 122 children, predominantly black, attended 5 urban day care centers. All had been enrolled for at least 4 months. The standard analyses, t test and Pearson's r were used to evaluate the urban-suburban differences and the correlation of blood lead with serum iron and other indices.

From the initial group of 154 children, 3 groups of iron deficient children without anemia were selected for evaluation of 1) the effects of increased blood lead on response to iron therapy and, 2) the effects of iron supplementation on blood lead. All children had a serum iron below 80 µg/dl and a hemoglobin above 10.5 gm%.

Group A: Low Fe, low Pb with fe supplementation: 14 children, blood lead below 17.5 µg/dl. Treated with oral ferrous sulfate, 200 mg/dl (40 mg elemental Fe or 1.15 - 1.75 mg/kg), given as a noon time dose at their school, 5 days per week for 4 months, March through June, 1974.

Group B: Low Fe, high Pb with Fe supplementation: 9 children, blood lead 17.5 - 40 µg/dl, iron supplementation as for Group A.

Group C: Low Fe, high Pb, no Fe supplementation: 7 children, blood lead 17.5 - 40 µg/dl, given a "placebo" solution containing 2 mg of ferrous sulfate, 5 days per week for 4 months.

Matched pairs from Groups B and C:

Groups B and C initially consisted of 11 pairs of children matched for serum Fe $\pm$ 20 µg/dl and Pb-B $\pm$ 8 µg/dl. They received, in a double blind study, either 200 mg or 2 mg of ferrous sulfate in an oral solution, 5 days per week for 4 months. Two children were lost from the treated group and 4 from the matched group. Seven matched pairs, including 3 sets of sibs, completed the study and analysis of the response employed the Wilcoxen signed ranks test.

The 30 children in Groups A, B, and C had a repeat of the initial studies within 4 weeks of completion of the oral iron supplement.

Seasonal Sampling

The January to July sampling was partly convenience and partly an attempt to modify the known seasonal changes in both serum iron and blood lead. Serum iron is highest in the first half of the year with a peak in March[45]. Blood lead is highest in the second half of the year with a peak in August. This relationship is shown in a graph of data from Dawson et al[46] of the seasonal changes in placental Pb, Ca, and Fe (Figure 6).

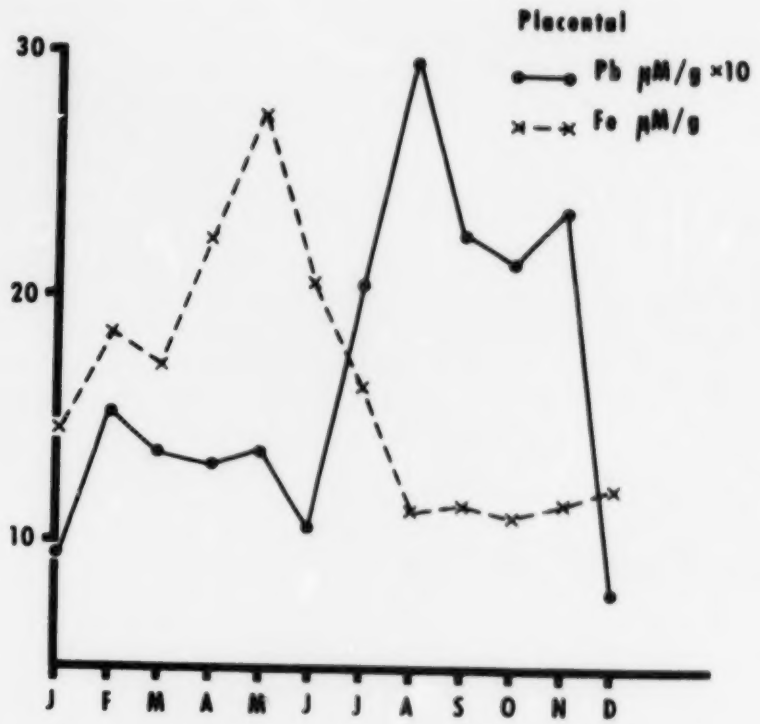

Figure 6: Seasonal variations in the placental concentrations of Pb (μM/g x 10) and Fe (μM/g) adapted from data by Dawson et al[46].

C. RED CELL METABOLISM

1. Enzymes of Red Cell Glycolysis and Cell Age

Samples of venous blood from 79 urban and suburban grade school and high school students, ages 10-18 (as cited in Section VI -A) and from 29 black males, ages 10-12, with G-6-PD deficiency, were assayed for 2,3 DPG by the method of Maeda[44], glucose-6-phosphate dehydrogenase (G-6-PD), 6-phosphogluonic dehydrogenase (6-PGD), and total glutathione, all by the methods of Beutler[47]. The G-6-PD deficient subjects all had less than 2.5 IU/gm Hgb and as black males were probably, although not proven, to have the A^- enzyme. Enzyme activity, hemoglobin, hematocrit, red cell indices, and reticulocytes were compared by t test for those with Pb-B below and above 20 µg/dl.

2. Lead Concentration in Young and Old Red Cells

Heparinized venous blood obtained from 4 healthy adults was incubated with lead at 100 µg/dl for one hour at room temperature. Differential centrifugation was carried out by the method of Murphy[48] in an angle-head rotor at 30° and 15,000 rpm for one hour. The top and bottom 10% of the red cells were separated and reconstituted with the subjects' own plasma to the original hematocrit and assayed for lead content by the micro method of Delves.

3. Assay of Na/K ATPase, Materials and Methods

Subjects -

Assays of red cell membrane Na/K ATPase were done on 65 samples obtained from 44 males and 3 females. While no differences in enzyme activity had previously been found in relation to sex, predominately male subjects were chosen, with the composition of the five groups as follows:

Preschool:	3 girls, 2 boys, all black, ages 2-4 years, attending an urban day care center. The children had no history of pica, no iron deficiency anemia, and normal G-6-PD activity.
School Age:	36 assays were done on the following 22 subjects: 7 urban black males, ages 9-12, all attending one school adjacent to a battery plant; 8 males, ages 14-18 at one urban high school (14 samples); 8 white males, ages 14-18, attending a suburban high school (15 samples).
G-6-PD:	14 black males, ages 6-12, attending 3 urban schools, all with G-6-PD deficiency. (less than 2.5 IU/gm Hgb[47]), but no anemia or reticulocytosis. All were presumed to have the A^- variant of the G-6-PD enzyme, but this was not definitively established.
Adults:	9 white males, ages 20-35, either medical students or laboratory staff.
Lead Workers:	2 white males employed in the lead refinery, no overt symptoms of lead poisoning.

Blood Lead -

All samples were drawn by venipuncture into lead free heparinized vacuum tubes. Whole blood samples were assayed for blood lead by the macro method of Farrelly and Pybus[42]. Blood lead (Pb-B) is reported as micrograms/deciliter (µg/dl).

Red Cell Membrane Na/K ATPase, Membrane Preparation -

Membrane fragments were prepared and assayed as described by Post[49,50], except that no EDTA was used. All glassware was acid washed and rinsed with water that was first distilled and then deionized. The red cells (RBC) from 5 ml of heparinized blood were washed 3 times with 6 volumes of 0.15 M NaCl, were centrifuged at 400 x g for 4 minutes and resuspended in NaCl. All subsequent procedures were done at 2° C. The RBC suspension was separated for duplicate determinations. One volume of cells was hemolyzed with 8 volumes of 2.2 mM imidazole, buffered with HCl to pH 6.8 $\pm$ 0.1, and was centrifuged at 20,000 x g for 10 minutes. This procedure was then repeated on the sediment. The sediment was then washed and centrifuged 4 times with 8.6 mM NaCl and 3.68 mM imidazole, pH 7.8 $\pm$ 0.1. The fluffy sediment, consisting of red cell ghosts, was refrigerated and incubated overnight in 1.3 mM urea and then rewashed with the saline-imidazole solution at which time it would be verified that the ghosts were broken into membrane fragments. These membrane fragments were suspended in the saline-imidazole solution to the original volume and the enzyme was assayed immediately.

In Vitro Studies of ATPase Activity -

In vitro studies of ATPase activity were done by the same method employing blood samples from 4 subjects for the effect of preincubation for 1 hour at 37° of whole blood with Pb of 100 µg/dl before preparation of the membrane fragment and of the incubation of membrane fragments with lead under the same conditions.

SECTION VII

DEMONSTRATION OF QUANTITATIVE METHODS

A. ENVIRONMENTAL STUDIES

1. Urban-Suburban and Age Related Differences in Blood Lead

As defined in the following table, the urban-suburban differences in Pb-B were most extreme in preschool children and decreased with age:

Table 1. AGE AND URBAN-SUBURBAN DIFFERENCES IN BLOOD LEAD

Age	Urban	Suburban	p
1-5 years 1974	22.9 ± 0.6 (122)	14.4 ± 0.6 (31)	<.001
10-12 years 1973	21.7 ± 0.5 (35)	17.1 ± 0.7 (35)	<.01
14-18 years 1973	22.3 ± 1.2 (14)	20.2 ± 0.7 (23)	n.s.
14-18 years males only 1973	25.6 ± 2.8 (8)	20.7 ± 0.9 (17)	<.05

Blood lead of urban-suburban children. Each pair of groups was tested in same month and same methodology (micro or macro). Pb-B given as mean ± SE with number of subjects in parentheses. Students t used to test for significant differences.

The decrease in Pb-B with age and the lessening urban-suburban difference is diagrammed in Figure 7.

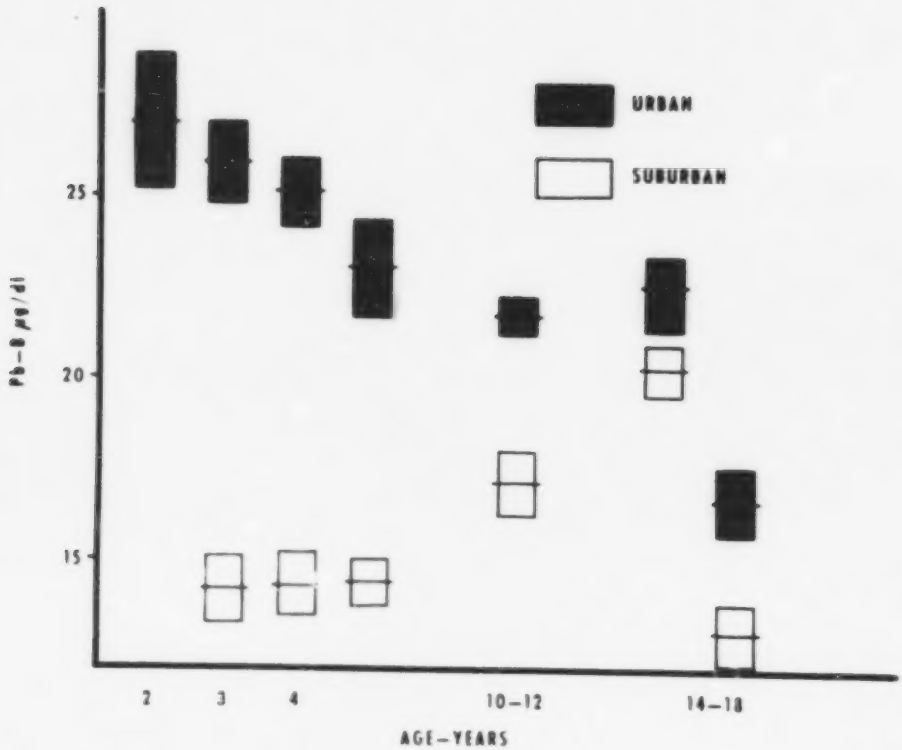

Figure 7. Pb-B (mean + SE) of representative samplings of urban and suburban children as listed in Table 1.

The decrease in Pb-B in urban children from ages 2 to 5 years is given in Table 2. A comparable decrease in blood lead from ages 2 to 5 years was not noted in the relatively small sample of suburban children:

Table 2. BLOOD LEAD, URBAN-SUBURBAN, AGES 2-5 YEARS

Age, years	Urban	Suburban
2	27.0 ± 1.9 (35)	-
3	25.9 ± 1.2 (70)	14.2* ± 0.9 (7)
4	25.1 ± 0.9 (71)	14.3* ± 0.9 (21)
5	23.1** ± 1.4 (34)	14.4* ± 0.5 (8)

Blood lead, µg/dl, mean ± SE, in urban and suburban preschool children in Omaha, 1974.

* $p < .05$ by t test for urban-suburban difference.

** $p < .05$ for urban children age 2 vs age 5.

In the age group, 14-18 years, the mean Pb-B of the total group (male plus female) did not differ, but monthly measurements, over 3 to 5 months, showed a significantly higher mean blood lead in urban than suburban boys (25.0 vs 20.7; $p < .05$), as listed in Table 3:

Table 3. MONTHLY BLOOD LEAD URBAN-SUBURBAN MALES, 14-18 YEARS

	Urban	Suburban	p
1972 Dec.	27.0 ± 4.7 (8)	22.7 ± 2.3 (15)	n.s.
1973 Jan.	22.7 ± 3.6 (6)	17.0 ± 0.6 (16)	n.s.
Mar.	27.1 ± 3.1 (8)	20.1 ± 1.9 (14)	< .05
Apr.	31.4 ± 4.2 (8)	22.1 ± 1.5 (17)	< .01
May	21.1 ± 1.7 (8)	21.5 ± 0.9 (17)	n.s.
Mean	25.0 ± 2.8 (8)	20.7 ± 0.9 (17)	< .01

Monthly Pb-B, µg/dl, mean ± SE, of 8 urban and 17 suburban high school boys. Macro assay used in January and May, micro assay in other months. F ratio used for determination of significant difference.

Seasonal Changes in Blood Lead

The seasonal changes in Pb-B in males ages 14-18 have been given in Table 3. The seasonal changes from December, 1972 - May, 1973 in male and female students, 14-18 years old, are shown in Figure 8:

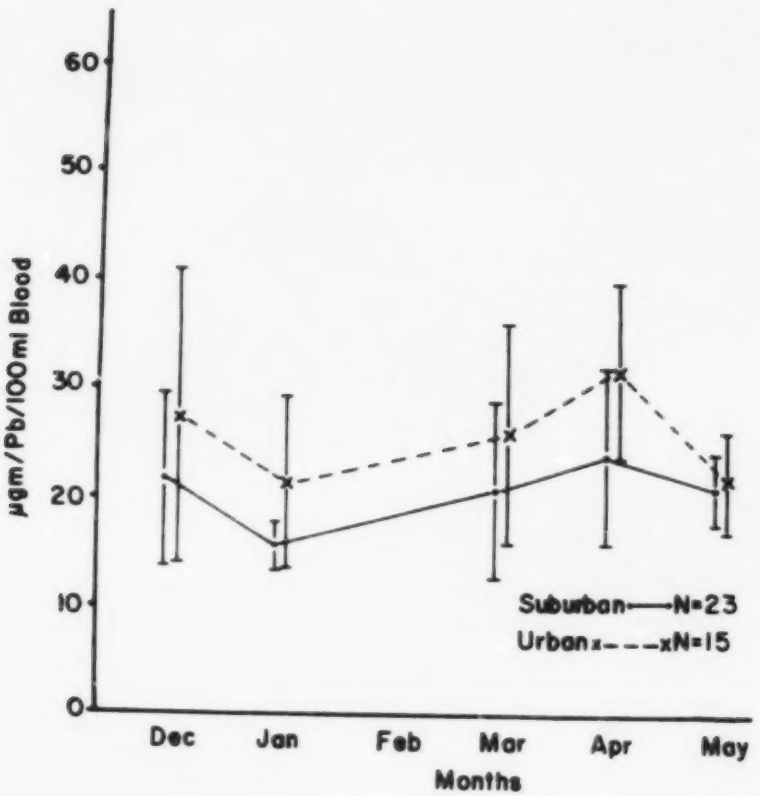

Figure 8. Monthly variations in Pb-B (mean ± SD) in 23 suburban and 15 urban students, ages 14-18. No on-line or lag-time correlation with air lead or dustfall lead could be demonstrated.

In the age group 2-5 years, repeat venous sampling for blood lead was done in 30 preschool children. The seasonal increase, given in Figure 9, is of similar magnitude in urban and suburban children. An increase from March to April, similar to the high school students, is also found in a small sample of urban children.

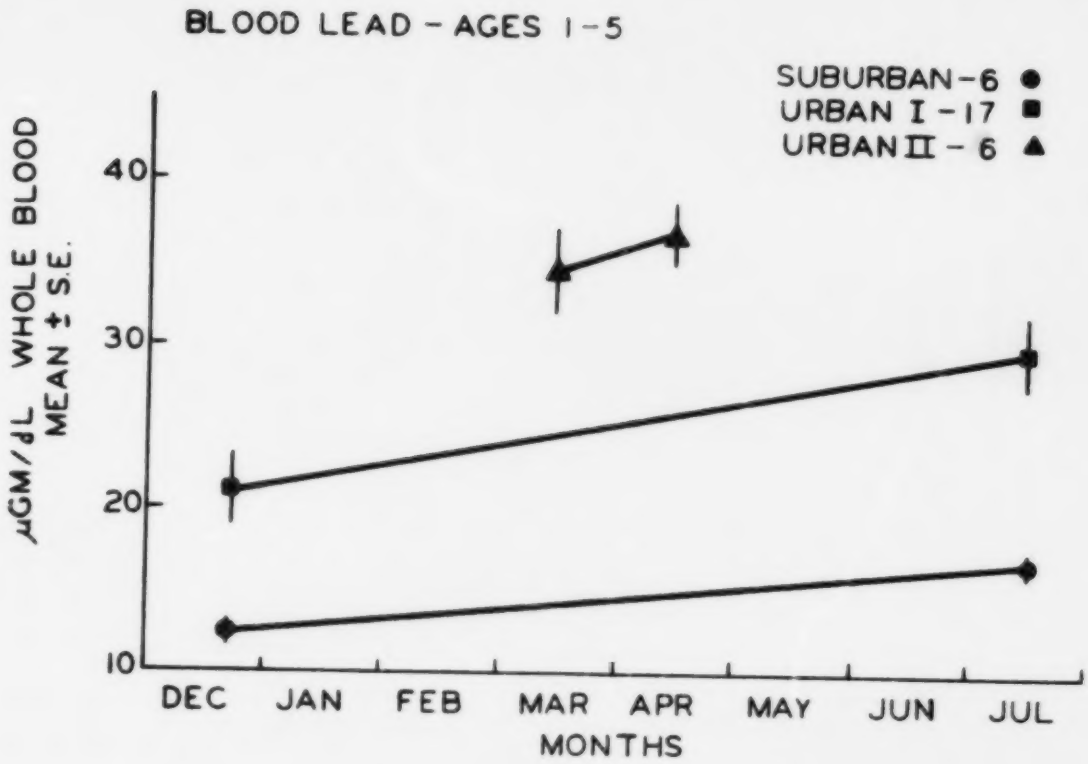

Figure 9. Seasonal change in Pb-B from December to July in urban and suburban children, ages 2-5, each with at least two serial venous samples. Blood lead is given µg/dl, mean ± SE.

2. Air Lead

The decline in air lead in Omaha over 5 years as monitored at 3 sites is shown in Figure 10. The highest values each year were found at the urban grade school which is located adjacent to a battery fabrication plant. In 1973, the year of the introduction of unleaded gasoline, there is a significant drop in suburban as well as urban air lead. City wide, industrial installation of antipollution devices began in 1971-72.

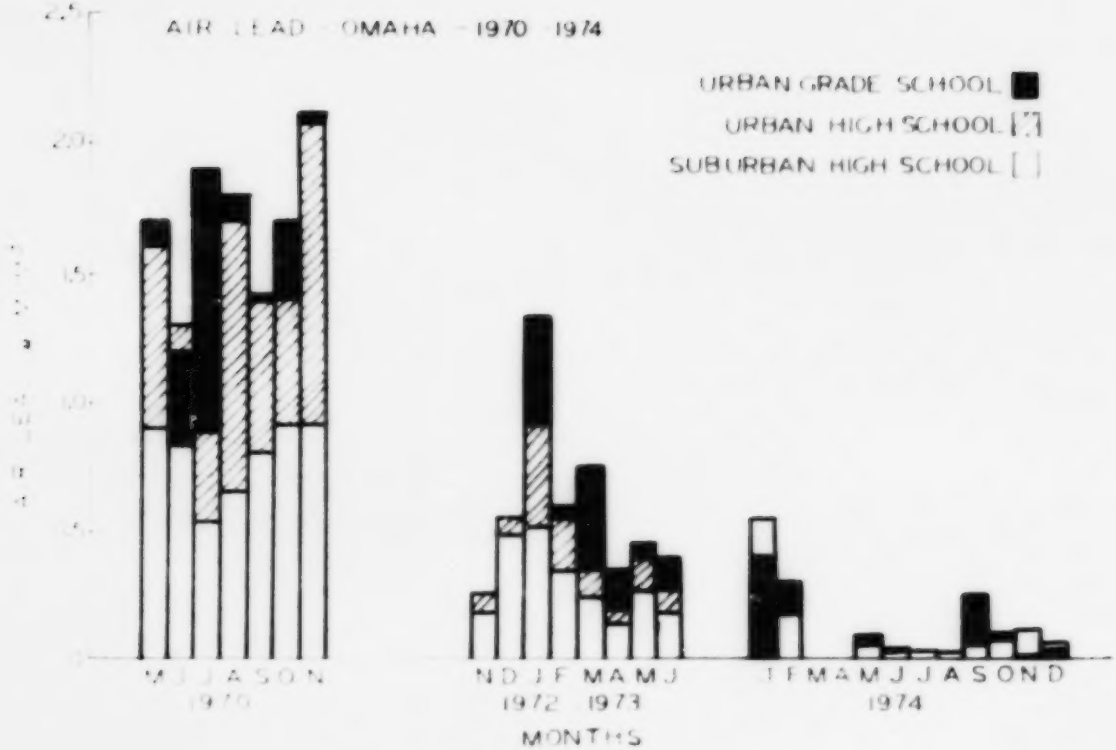
AIR LEAD - OMAHA - 1970 1974
URBAN GRADE SCHOOL
URBAN HIGH SCHOOL
SUBURBAN HIGH SCHOOL
M J J A S O N
1970
N D J F M A M J
1972 1973
J F M A M J J A S O N D
1974
MONTHS

Figure 10. Monthly mean air lead in Omaha 1970-1971 at 3 sites. The urban grade school site was at 24th and Pinkney in 1970 and 30th and Spaulding thereafter. The urban high school site (30th and Cuming) was constant. The suburban site was at 48th and O in 1970 and 88th and Pacific thereafter.

The annual mean composite air lead at the 3 sites is given in Table 4:

Table 4. COMPOSITE AIR LEAD IN OMAHA 1970-1974

	1970 May-Nov	1972-73 Nov-June	1974 Jan-Dec
Suburban 48th & Q, 1970 88th & Pacific, 1972-74	0.79	0.29	0.12
Urban high school 30th & Cuming	1.48	0.43	-
Urban 24th & Pinkney, 1970 30th & Sprague, 1972-74	1.69	0.63	0.10

Air lead $\mu g/M^3$, as the 7 to 10 months mean of the monthly means of 24-hour Hi Vol collections at 3 sites.

Because of the possibility of higher air leads at the nearground level of air intake of the young child, air lead was monitored at both 3' and 15' at the urban grade school. The correlation of monthly values was significant at $r = 0.94$ ($p < .05$), but no significant difference was found between air lead at the two elevations, as shown in Figure 11:

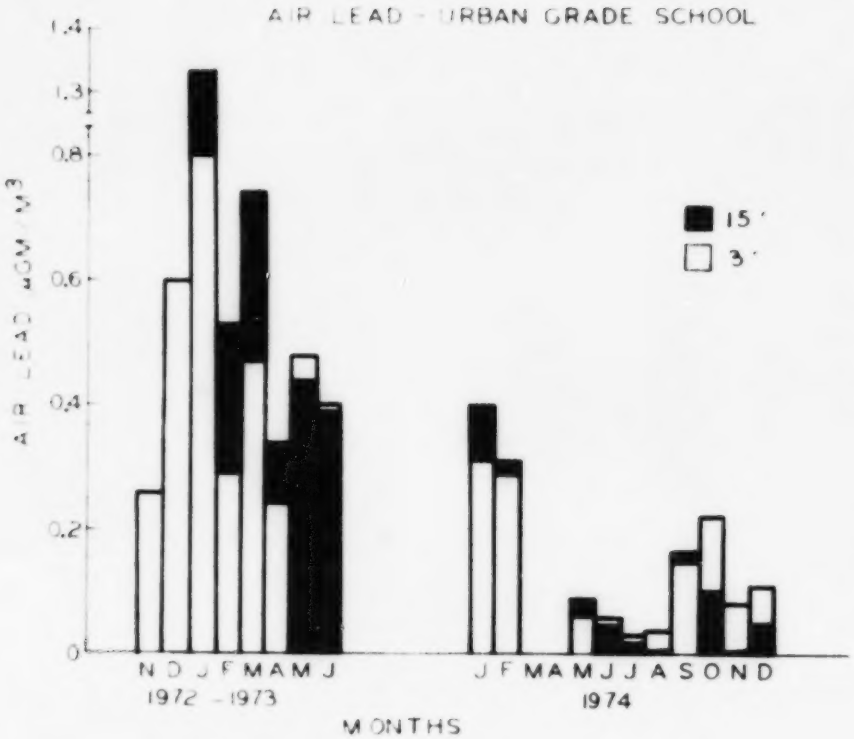

Figure 11. Air lead at 3' and 15' elevation. Air lead in $\mu g/M^3$, as the monthly mean of 6 to 20 random 24-hour collections per month taken in Hi-Vol samplers at 3' and 15' feet elevation at the urban grade school (30th & Sprague). There is no significant difference in the 16 months mean at 3' (0.25 $\mu g/M^3$) and at 15' (0.32 $\mu g/M^3$).

3. Dustfall Lead

A significant decline was noted in dustfall lead as collected in 30 day samples at the same 3 sites as the air lead from March, 1973 through April, 1974 (Figure 12). Unlike air lead, urban-suburban differences in the dustfall lead were still significant in 1974 with maximum levels at the school adjacent to the battery plant.

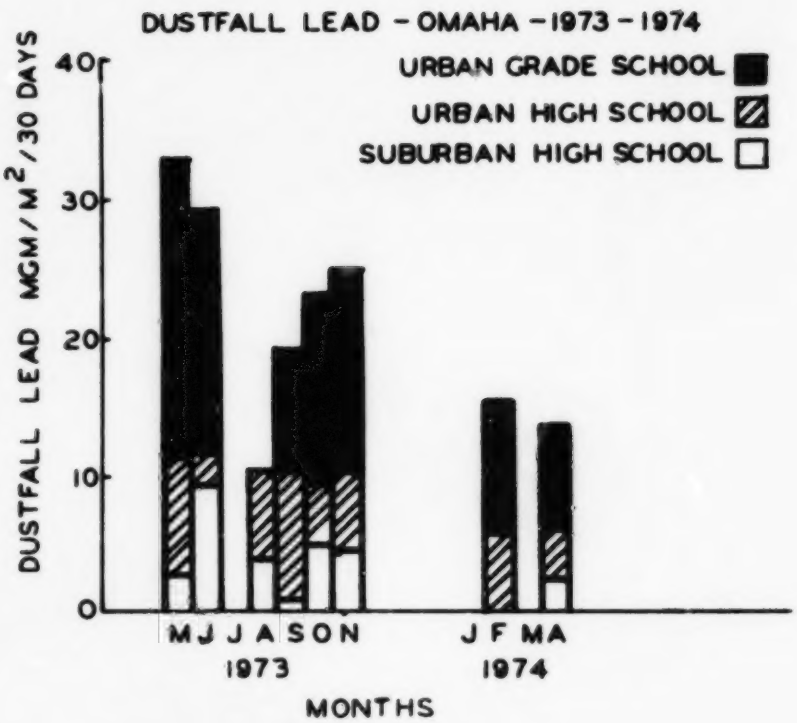

Figure 12. Dustfall lead as $mg/M^2/30$ day collection at 3 sites. The mean dustfall lead at the urban grade school for 9 months was 22.7 ± 2.7 $mg/M^2/30$ days, significantly higher ($p < .01$) than the mean values at the urban high school (9.4 ± 0.8) and the suburban high school (4.3 ± 0.9).

Lead in Exterior Soil and Housedust

Exterior soil samples (4 at each site) were obtained from 4 schools and 25 homes in 1973, and from 7 day care centers in 1974 for a total of 154 assays. Exterior soil from urban areas had a lead content ranging from 100-2400 µg/gm; all suburban samples had less than 400 µg/gm.

Housedust was obtained from 34 homes and 7 day care centers. The urban values for lead ranged from 50 to 1200 µg/gm, suburban samples contained 110 to 610 µg/gm.

Boot tray dust collections, representing a composite of exterior and interior soil, had a lead content between 90 and 2400 µg/gm in 14 urban samples and 50 to 500 µg/gm in 9 suburban samples.

Lead in Milk and Water

Water, collected as a composite 1 month sample from 20 homes in 1973, never had a lead content above .01 µg/ml. The lead content of 20 milk samples was between .02 and .03 µg/ml.

4. Correlation of Environmental Lead and Blood Lead

The higher Pb-B of urban than suburban preschool children was associated with a significantly ($p < .001$) higher dustfall lead and interior dust ($p < .05$), as shown in Table 5. The relation of the general environmental sources to Pb-B is shown graphically in Figure 13.

Table 5. ENVIRONMENTAL: BLOOD LEAD, AGES 2-5, 1974

	Suburban	Urban	p
Pb-B	14.4 ± 0.6 (31)	22.3 ± 0.6 (122)	< .001
Air Pb µg/M^3	0.14 ± .02	0.12 ± .05	n.s
Dustfall Pb, mg/M^2/mo	2.94 ± 0.91	20.47 ± 2.72	< .001
		7.05 ± 1.19	< .01
Interior dust Pb, µg/gm	175 ± 60 (2)	550 ± 155 (5)	< .05
Yard dirt Pb, µg/gm	97 ± 84 (7)	219 ± 105 (20)	n.s.

Blood lead of 31 suburban and 122 urban children in 1974 with comparison of the lead content of general environmental sources. All values given as mean ± SE. Numbers in parentheses are number of subjects or of samples. Urban dustfall at each of the 2 urban sites of Figure 1, is significantly higher than the suburban dustfall.

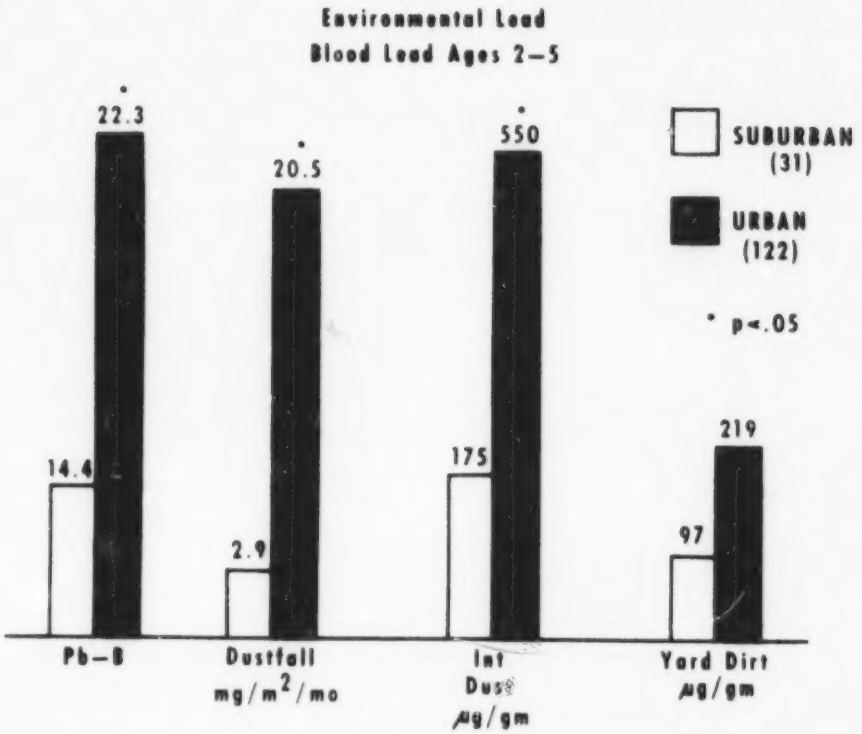

Figure 13. Environmental Pb and Pb-B, ages 2-5, of 122 children at 5 urban day care centers and 31 children at 2 suburban centers. The children at the urban centers have significantly higher mean Pb-B, 22.3 ± 0.6 vs 14.4 ± 0.6 µg/dl ($p < .001$), associated with a higher dustfall lead, 20.5 ± 2.7 vs 2.9 ± 0.9 ($p < .001$), and interior dust lead 550 ± 155 vs 175 ± 60 µg/gm ($p < .05$). All values as mean ± SE.

In the grade school students, ages 10-12, studied in 1973, the higher level of Pb-B in the urban group was associated with a higher dustfall lead as shown in Table 6, and plotted in Figure 14.

Table 6. ENVIRONMENTAL AND BLOOD LEAD, AGES 10-12

	Suburban	Urban
Number of subjects	35	35
Pb-B (micro), µg/dl	17.1 ± 0.7	21.7 ± 0.5
Air Pb, $\mu g/M^3$	0.29	0.63
Dustfall, $mg/M^2/mo$	3.05	32.96
Milk, µg/ml	< .04	< .04
Water, µg/ml	< .01	< .01

Blood lead (mean ± SE) of 35 urban and 35 suburban studies by micro assay, fingerstick sample of January, 1973.
Air lead is the mean for January, 1973 and dustfall lead is the mean for April - May, 1973. Values for milk and water were the means of a single milk and a one month composite water sample collected in April and May, 1973 at the homes of 5 urban and 5 suburban children of each group.

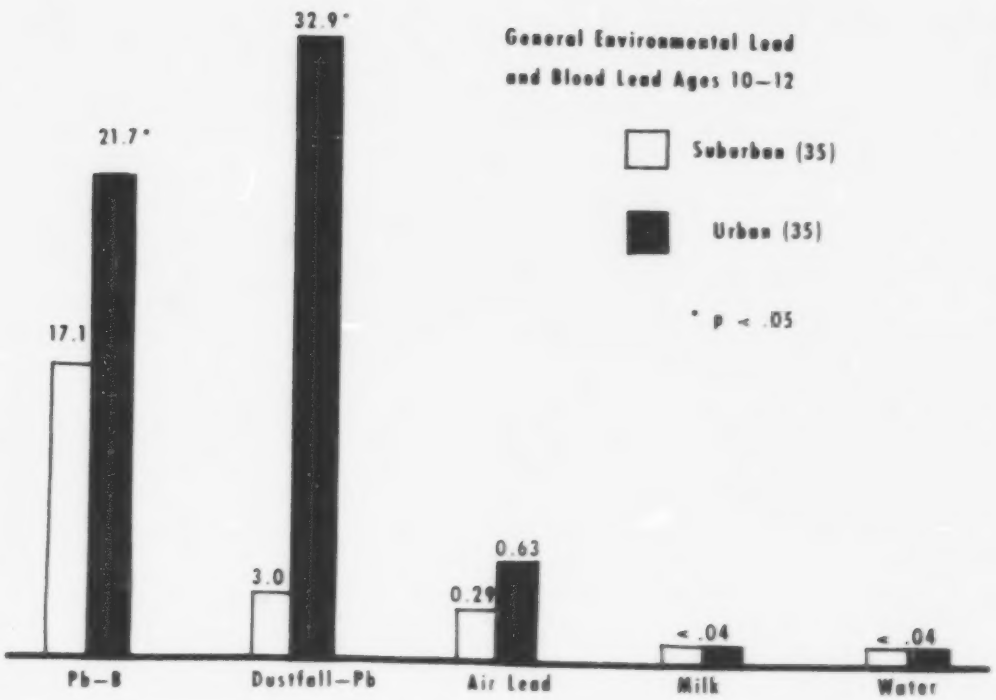

Figure 14. Environmental Pb and Pb-B, ages 10-12. There is a significant ($p < .05$ by t test) difference in Pb-B, dustfall and soil lead with no significant difference in lead in milk or water.

In the individual homes, there was a significant difference in the lead content of soil and boot tray dirt as shown in Table 7 and Figure 15.

Table 7. HOUSEHOLD LEAD AND BLOOD LEAD, AGES 10-12

	Suburban	Urban	
Number	9	16	
Pb-B, micro	17.7 ± 2.3	22.4 ± 2.4	
Soil, mean, ppm	123 ± 28	444 ± 62	p<.005*
Boot tray, ppm	283 ±157	1001 ± 472	p<.05**
Housedust, ppm	335 ± 68	572 ± 147	
Paint chips, > 1000 ppm	1/9	1/16	

* t test.

** $\bar{z}$ ratio for uncorrelated proportions of values greater than the group median of 370 ppm.

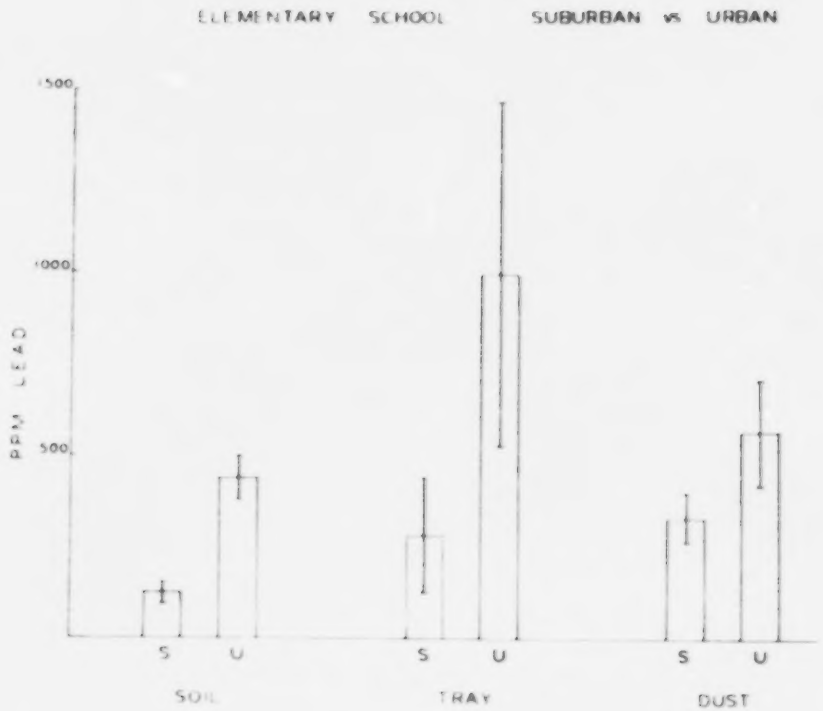

Figure 15. Household lead, urban (U) and suburban (S). Lead content, µg/gm as mean ± SE, as measured at the homes of 9 suburban and 16 urban children, in yard dirt, boot tray collections, and house dust, as given in Table 7.

Within the total group of 16 urban students, ages 10-12, those with Pb-B above 20 µg/dl had significantly higher levels of lead in yard dirt and boot tray dirt as shown in Table 8.

Table 8. HOUSEHOLD LEAD - CHILDREN 10-12 WITH HIGH AND LOW BLOOD LEAD

	Pb-B < 20	Pb-B ≥ 20
Number of subjects	7	9
Pb-B, µg/dl	15.5 ± 1.4	27.4 ± 1.4**
Soil, mean, ppm	346 ± 94	519 ± 79 **
Boot tray, ppm	470 ± 141	1442 ± 847*
Housedust, ppm	449 ± 108	692 ± 278
Paint chips	0	1/9 (93,636 ppm)

All values given as the mean ± SE.
** $p < .005$, * $p < .02$ by t test.

There was a significant correlation ($r = .49$; $p < .05$) of Pb-B with lead in yard dirt in this age group (Table 9). Correlation Pb-B with lead in boot tray dirt was positive (0.23) but not significant for 16 subjects. There was no correlation of Pb-B with lead in house dust, milk or household water for ages 10-12 years.

Table 9. HOUSEHOLD LEAD, CORRELATION WITH BLOOD LEAD, AGES 10-12

Urban, ages 10-12	r
Soil	.49 ($p < .05$)
Boot tray	.23
Housedust	.01
Milk	.00
Water	.00

In urban males of high school age, the higher blood lead (mean of serial measurements over 5 months) was associated with an urban dustfall lead that was almost 3 times the suburban value (Table 10). Air lead in the two areas was not significantly different. Lead in milk and water was low in all samples in both areas.

Table 10. ENVIRONMENTAL AND BLOOD LEAD, MALES, AGES 14-18

December, 1972 - May, 1973

	Suburban	Urban
Number of subjects	17	8
Pb-B x 5, µg/dl	20.7 ± 0.9	25.0* ± 2.8
Air Pb, µg/M^3, Nov - June	0.29	0.43
Dustfall, mg/M^2/mo Apr - May	3.0	11.4**
Milk, µg/ml	< .04	< .04
Water, µg/ml	< .01	< .01

* $p < .05$; ** $p < .01$ by t test.

In the study of 15 individual homes, the homes of 8 urban high school students (Pb-B 16.3), had significantly higher lead in yard dirt than did the homes of 7 suburban students (Pb-B 13.1), as listed in Table 11.

Table 11. HOUSEHOLD LEAD AND BLOOD LEAD, AGES 14-18, 1973

	Suburban	Urban
Number of subjects	7	8
Pb-B, µg/dl	13.1	16.3
Soil, mean, ppm	89.6 ± 10.8	863.5 ± 243 *
Roof tray, ppm	196.0 ± 80.9	254.2 ± 104
Housedust, ppm	339.6 ± 77.5	361.3 ± 36.4

* $p < .02$ by t test. Values given as mean ± SE.

In the high school age, correlation of Pb-B with lead in soil and house dust was positive although not statistically significant for the small size of the sample as shown in Table 12. Correlation of Pb-B with lead in yard dirt ($r = 0.31$) and with lead in house dust ($r = 0.29$) was similar.

Table 12. HOUSEHOLD LEAD, CORRELATION WITH BLOOD LEAD, AGES 14-18

	r
Soil	.31
Housedust	.29
Boot tray	-.04
Milk	.00
Water	.00

Proximity to Traffic as Related to Blood Lead and Soil Lead -

The home address of each of the urban students, ages 10-18, was plotted on a metropolitan map and the distance from a major trafficway was plotted. In the northcentral area of Omaha, 6 trafficways are defined by the City Traffic Department as carrying more than 3500 vehicles per lane per day. As shown in Table 13, the 9 students living within 750' of a trafficway had a significantly higher mean Pb-B than the 18 students living at or beyond 750' (27.3 vs 21.0; $p < .02$).

Table 13. PROXIMITY TO TRAFFIC AND BLOOD LEAD, URBAN STUDENTS, AGES 10-18

	< 750'	> 750'
Number of subjects	9	18
Pb-B, ug/dl	27.3 ± 2.6	21.04 ± 1.3*

The households of 27 urban students, ages 10-18, were categorized as within or beyond 750 feet from a street carrying more than 3500 cars/lane/day. The difference in Pb-B is significant.
* $p < .02$, Student's t test.

In the group of 24 urban students, ages 10-12 and 14-18, the best correlation of Pb-B with an environmental source was with lead in yard dirt. The lead content of yard dirt, in turn, was linearly related ($r = -.42$) to the residential distance from traffic as shown in Figure 16.

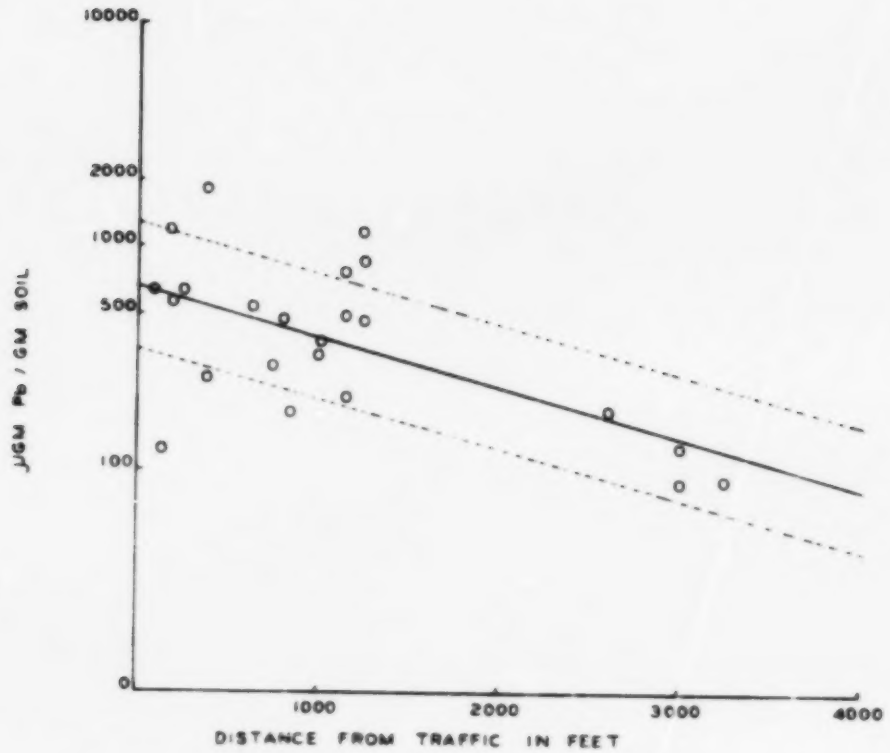

Figure 16. Lead in yard dirt and residential distance from traffic. There is a significant correlation ($r = -.42$; $p < .05$) of the lead content of yard dirt (mean of 4 samples) with residential proximity to traffic for the houses of 24 urban students, ages 10-18, studied in 1973. Traffic is defined as any of the streets in northcentral Omaha with over 3500 vehicles/lane/day.

B. LEAD AND IRON DEFICIENCY

Urban-Suburban Differences in Blood Lead, Hematologic Indices and 2,3 DPG:

As shown in Table 14, the urban black preschool children had significantly higher Pb-B (22.3 vs 14.4 µg/dl) and lower hemoglobins (12.3 vs 12.7 gm%) than their suburban counterparts. There was no difference in serum iron (Fe), iron binding capacity (TIBC), or percent saturation of iron binding capacity (% Fe Sat).

Table 14. BLOOD LEAD AND HEMATOLOGIC INDICES

Urban-Suburban Differences

Ages 2-5 Years

	Urban	Suburban	p
Pb-B, µg/dl	22.3 ± 0.6 (122)	14.4 ± 0.6 (31)	< .001
Hgb, gm%	12.3 ± 0.07 (122)	12.7 ± 0.13 (31)	< .01
Hct, %	36.7 ± 0.2 (122)	37.4 ± 0.3 (31)	n.s.
Fe	93 ± 6 (107)	94 ± 3 (31)	n.s.
TIBC, µg/dl	363 ± 6 (102)	366 ± 8 (31)	n.s.
% Fe Sat.	25.3 ± 0.8 (101)	25.4 ± 1.3 (31)	n.s.
2,3 DPG, µM/L	4.70 ± 0.11 (103)	3.95 ± 0.16 (31)	< .001

All values given as mean ± SE; numbers of subjects in parentheses; p derived from Student's t test.

The urban children also had significantly higher concentrations of red cell 2,3 DPG (4.70 vs 3.95 μM/L), an observation not completely explained by the differences in hemoglobin. Correlation of 2,3 DPG with hemoglobin was somewhat higher, $r = 0.36$; $p < .05$, in the 31 suburban children than in the 103 urban children where $r = 0.20$; $p < .05$. The serum phosphorus was assayed in 42 preschool children and in 53 children, ages 10-12, to see if this accounted for the differences in 2,3 DPG. The resulting intercorrelations are shown in Table 15. Serum 2,3 DPG again showed the expected inverse correlation with hemoglobin, but was unrelated to Pb-B. The serum phosphorus was correlated ($r = 0.2$) with blood lead, a phenomenon probably related to the higher blood lead and higher serum phosphorus of the younger children.

Table 15. 2,3 DPG INTERCORRELATIONS, AGES 2-12

	Hgb	Phos	Pb-B	Fe
2,3 DPG	-.31*	.14	0.12	.09
Hgb		-0.32*	-.16	.28*
Phos			.20*	-
Pb-B				.22*

*$p < .05$

Correlation of Pb-B with Hematologic Indices

Although the urban children had higher Pb-B and lower hemoglobin than suburban children, within the group of urban preschool children there was a significant positive correlation of Pb-B with Fe and with hematocrit. Serum Fe and TIBC correlated with hematocrit and hemoglobin, and 2,3 DPG had the expected negative correlation with hemoglobin and hematocrit (Table 16).

Table 16. CORRELATIONS OF BLOOD LEAD, SERUM IRON, AND HEMATOLOGIC INDICES

Urban Children, Ages 2-5 Years

	Fe	% Fe Sat.	Hgb	Hct	2,3 DPG
Pb-B	+	+	+*	+	0
Fe		+	+	+	0
% Fe Sat.			+	+	0
Hgb				+	-
Hct					-

Significant ($p < 0.05$) correlations are labelled as positive (+) or negative (-). Correlations not significant at the 95th percentile are labelled 0. * The correlation of Pb-B with Hgb was positive, $r = 0.19$, although significant at only $p < 0.1$.

Hematologic Indices in Fe Deficiency with High and Low Pb-B

The possible augmentation of Fe deficiency anemia by moderate elevation of the Pb-B was investigated by comparison of the hematologic indices of iron deficient children (Fe < 80) with Pb-B below and above 20 µg/dl. There was no significant difference in the mean Hgb, Hct, MCV, MCH, MCHC, or 2,3 DPG of the 29 Fe deficient children who had Pb-B < 20 µg/dl and that of the 17 Fe deficient children with Pb-B 20-40 µg/dl.

Serum Fe Response with High and Low Pb-B

The therapeutic response of serum Fe of 14 low lead children in Group A (Fe < 80, Pb-B < 17.5) to 4 months of oral $FeSo_4$ is compared with that of the 9 in the high lead Group B (Fe< 80, Pb-B 17.5 - 40) in Figure 17. The increase of the serum iron was almost identical in Group A where Pb-B increased from 13.9 $\pm$ 0.5 to 20.9 $\pm$ 1.5 µg/dl and in Group B, where Pb-B increased from 18.4 $\pm$ 1.6 to 25.7 $\pm$ 1.8 µg/dl.

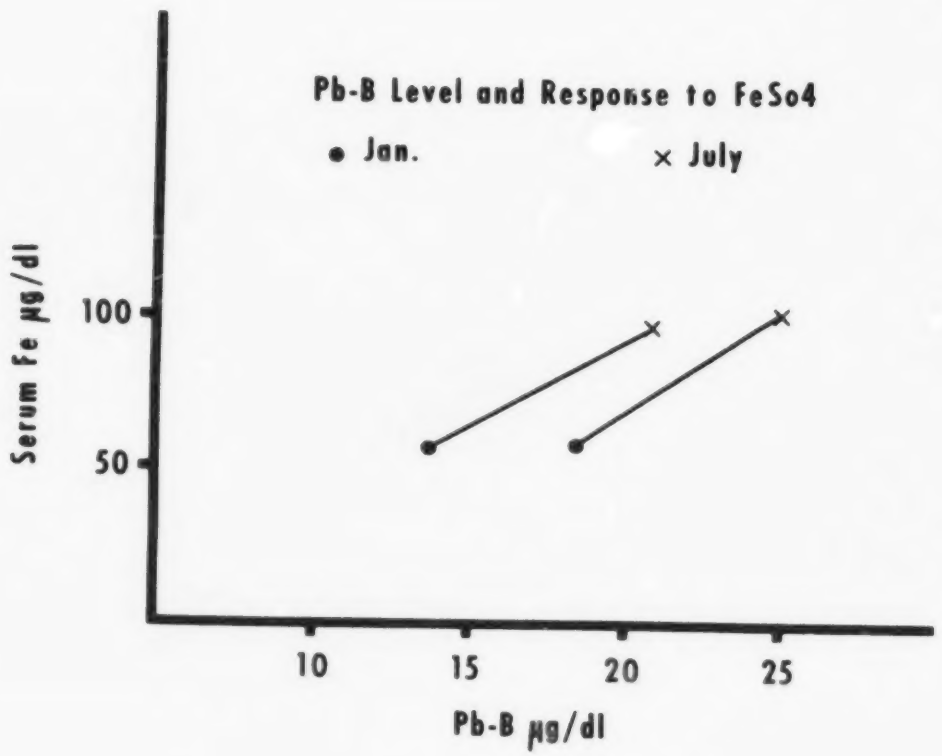

Figure 17. Response of serum Fe of Group A (N = 14, Fe < 80, Pb-B < 17.5) and Group B (N = 9, Fe < 80, Pb-B < 17.5) after 4 months of oral Fe therapy.

Serum Iron and Hemoglobin: Response to Therapy vs Placebo

The response to oral iron supplementation was compared for the 23 children who received 200 mg $FeSo_4$, 5 days per week, March through June (Groups A & B) and for the 7 children who received only 2 mg $FeSo_4$ for the same time (Group C). Although the iron treated children had slightly higher levels of iron and hemoglobin, neither of these differences was significant (Figures 18 and 19).

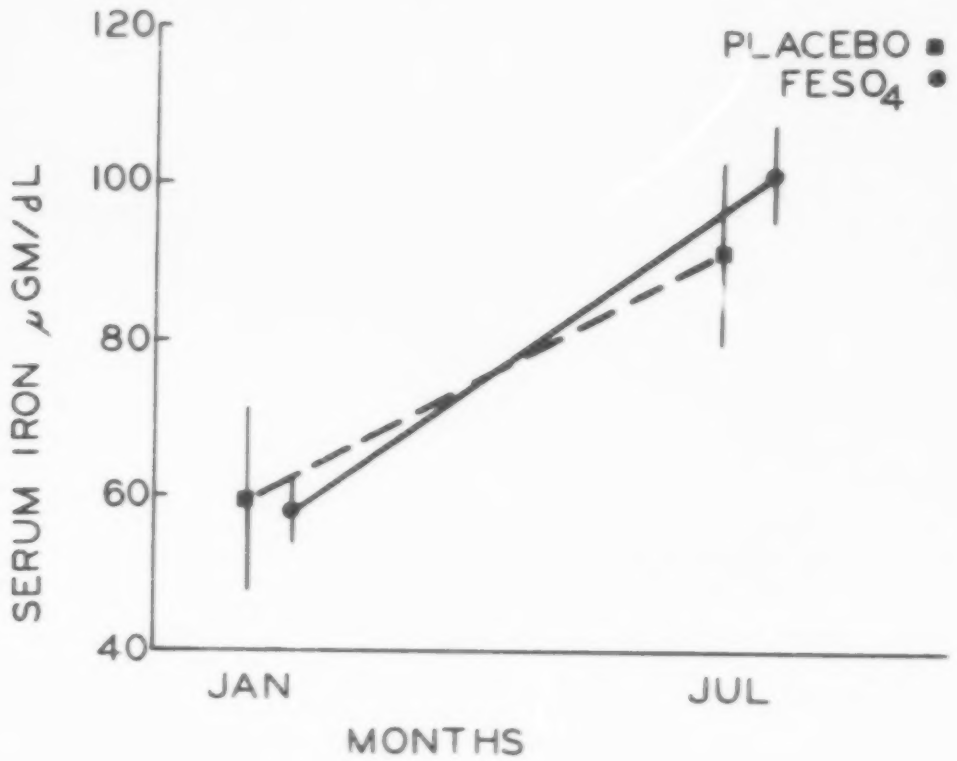

Figure 18. Oral iron vs placebo: January to July change in serum Fe (mean ± SE) in 23 children receiving $FeSo_4$, 200 mg/d, 5 days per week, March through June, and in 7 children receiving placebo.

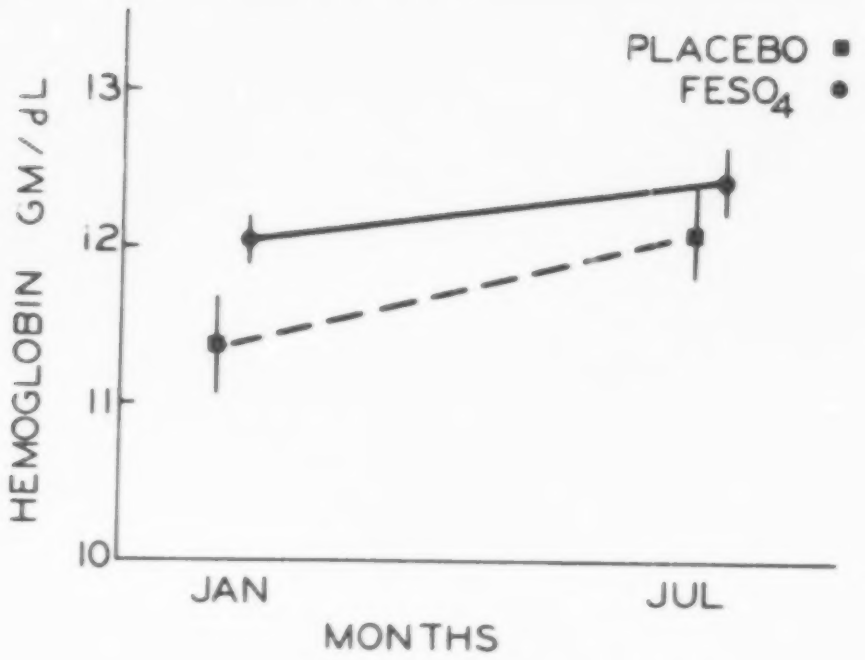

Figure 19. Oral iron vs placebo: January to July change in Hgb (mean ± SE) in $FeSo_4$ treated (N = 23) and placebo (N = 7).

Effect of Iron Supplementation on Blood Lead

Comparison of the seasonal change in Pb-B of the 14 low lead (Group A) and 9 high lead children (Group B) given 200 mg $FeSo_4$ with that of the 7 receiving only a 2 mg dose (Group C) for 4 months, as graphed in Figure 20, showed no difference in the seasonal increase of the blood lead.

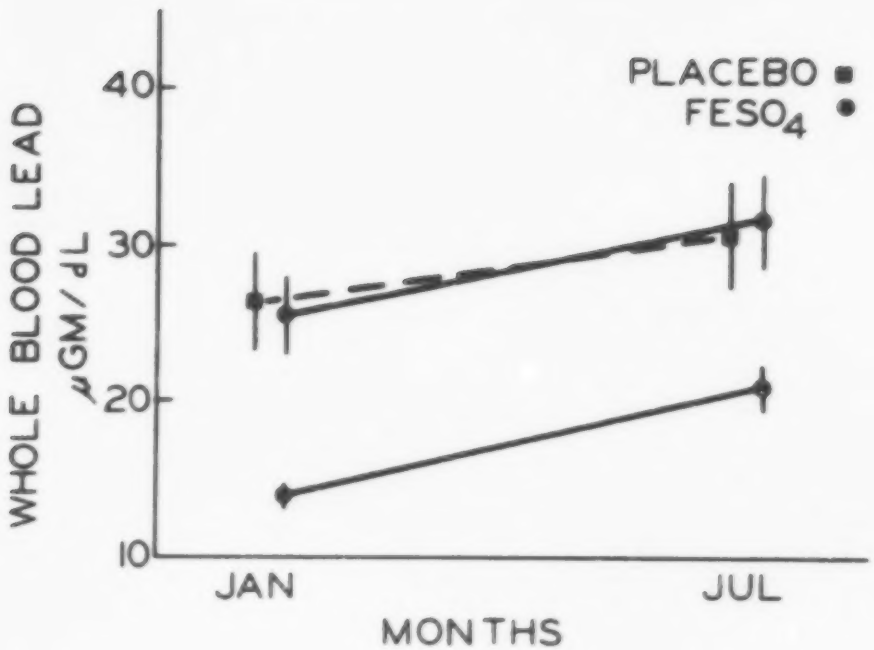

Figure 20. Oral iron vs placebo: change in blood lead. January to July change in Pb-B in 9 high lead and 14 low lead children treated with $FeSO_4$, March through June, and in 7 children receiving placebo.

The data comparing the entire treated group (A and B) with the placebo Group C is given in Table 17:

Table 17. Fe THERAPY VS PLACEBO: EFFECT ON SERUM Fe AND Pb-B IN IRON DEFICIENCY

Group	Serum Fe			Pb-B		
	Jan	July	Δ	Jan	July	Δ
A & B $FeSo_4$, 200 mg x 4 months N = 23	57.7 ± 3.5	101.1 ± 6.5	42.6 ± 6.7	18.4 ± 1.6	25.1 ± 1.8	6.7 ±1.1
C $FeSo_4$, 2 mg/d x 4 months N = 7	59.4 ±12.2	91.6 ±11.6	33.9 ±13.1	26.4 ± 3.0	30.7 ± 3.4	4.2 ±4.2
Significant Difference $p < .05$*	No	No	No	Yes	No	No

Serum iron (Fe), blood lead (Pb-B), and the January to July difference (Δ), all as µg/dl, mean ± SE in preschool children given oral ferrous sulfate, 200 mg or 2 mg/day, March through June.
* A+B vs C.

Table 18 details the response of 7 pairs of children matched for initial levels of serum Fe (± 20) and Pb-B (± 8) to the double blind administration of either 200 mg or 2 mg of $FeSo_4$ given at noon, 5 days per week for the four months, March through June. In 6 of 7 pairs, the seasonal increase in lead was greater in the treated group ($p = .06$ by sign test for matched pairs). Iron therapy was associated with a greater decrease in serum transferrin in 5 of 6 pairs and with a greater increase in hemoglobin in 5 of 5 pairs.

Table 18. BLOOD LEAD AND IRON THERAPY

Seasonal Response in 7 Pairs with Double Blind Administration of Fe or Placebo (P)

	Pb-B µg/dl		Fe µg/dl		CIBC µg/dl		Hab gm%	
	Jan	June	Jan	June	Jan	June	Jan	June
1. Fe	39.1	38.2	89	90	316	286	11.2	10.7
P	41.5	39.2	119	88	326	296	11.3	10.4
Δ Fe - Δ P	+ 1.4		-20		-62		+0.4	
2. Fe	20.6	23.3	38	132	284	338	-	12.4
P	22.3	23.4	58	123	276	292	11.8	13.2
Δ Fe - Δ P	+ 1.6		+29		+32			
3. Fe	22.7	24.9	66	86	398	320	11.9	13.7
P	28.8	29.0	68	146	342	324	11.6	12.5
Δ Fe - Δ P	+ 2.0		-58		-50		+0.9	
4. Fe	21.3	24.3	23	51	312	256	11.1	11.9
P	30.8	30.2	23	72	274	320	12.1	12.5
Δ Fe - Δ P	+ 3.6		-21		-102		+0.4	

Table 18 (continued). BLOOD LEAD AND IRON THERAPY

	Pb-B µg/dl		Fe µg/dl		TIBC µg/dl		Hgb gm%	
	Jan	June	Jan	June	Jan	June	Jan	June
5. Fe	29.6	37.5	59	63	346	278	12.9	13.3
P	21.6	26.5	50	74	272	250	12.0	11.7
Δ Fe - Δ P	+ 3.3		-20		-36		+0.7	
6. Fe	24.5	29.4	72	151	336	258	11.6	12.8
P	23.0	20.4	71	74	328	222	11.2	12.0
Δ Fe - Δ P	+ 7.5		+76		-174		+0.4	
7. Fe	17.5	20.1	27	66	196	-	12.9	-
P	17.1	46.1	36	-	336	254	9.5	11.6
Δ Fe - Δ P	-26.4							
Δ Fe > Δ P	6/7		2/6		1/6		5/5	
p	0.062		n.s.		0.109		0.031	

C. RED CELL METABOLISM

1. Enzymes of Red Cell Glycolysis

G-6-PD, 6-PGD, and GSH are of higher concentration in young red cells than in older cells and are recognized as indices of cell age [26,27]. 2,3 DPG is not a reliable index of cell age and varies with multiple factors such as hemoglobin, oxygen saturation and serum phosphorus. The data in Table 19, is from 79 students, ages 10-18, without G-6-PD deficiency with Pb-B above and below 20 µg/dl. There is a significant increase of G-6-PD and 6-PGD and a significant decrease in GSH in those subjects with a Pb-B $\geq$ 20 µg/dl. There was no significant difference in 2,3 DPG, hematocrit, hemoglobin, red cell indices, reticulocytes, or haptoglobins between the high and low lead group.

Table 19. BLOOD LEAD AND RBC ENZYMES REFLECTING CELL AGE, AGES 10-18

	Pb-B $<$ 20	Pb-B $\geq$ 20	p
G-6-PD	9.68 $\pm$ 0.20 (67)	11.08 $\pm$ 0.68 (11)	$<$ .05
6-PGD	7.57 $\pm$ 0.16 (67)	8.50 $\pm$ 0.43 (11)	$<$ .05
GSH	6.11 $\pm$ 0.14 (68)	5.56 $\pm$ 0.28 (11)	$<$ .05
HCT	42.2 $\pm$ 0.36 (68)	41.8 $\pm$ 0.61 (11)	n.s.
2,3 DPG	5.08 $\pm$ 0.16	5.30 $\pm$ 0.37	0.1

The values in this and the next two tables are expressed as 2,3 DPG µM/ml rbc[44], G-6-PD, and 6-PGD U/gm Hgb[17], and GSH µM/gm Hgb, mean $\pm$ SE.

Since GSH is normally increased in the young red cell, the decrease in these subjects suggests a specific depression of GSH by lead, as noted previously in lead workers with increased Pb-B[51-52].

In 29 black males, ages 10-12, with G-6-PD deficiency, the difference in the enzyme activity between those with Pb-B below and above 20 µg/dl, as shown in Table 20, was not significant. The levels of G-6-PD, 6-PGD and GSH are intrinsically lower in G-6-PD deficiency and were not stimulated by modest increases in Pb-B. There was no significant difference in hemoglobin, hematocrit, red cell indices, reticulocytes or haptoglobins between the low lead and high lead G-6-PD deficient boys.

Table 20. BLOOD LEAD AND RBC ENZYMES REFLECTING CELL AGE

G-6-PD Deficient Black Males, Ages 10-12

	Pb-B < 20	Pb-B ≥ 20
G-6-PD	0.89 ± 0.14 (13)	0.69 ± 0.12 (16)
6-PGD	8.63 ± 0.49 (13)	8.72 ± 0.46 (16)
GSH	4.39 ± 0.19 (13)	4.26 ± 0.18 (16)
2,3 DPG	5.26 ± 0.36 (12)	5.48 ± 0.25 (15)

All values given as mean $\pm$ SE in the same units as Table 19. No significant difference in mean of the two groups on 1 tailed t test. Number of subjects in parentheses.

Affinity of Lead for young and Old Red Cells

The affinity of lead, on in vitro incubation of whole blood with lead at 100 µg/dl, for the top 10% (young cells) and bottom 10% (old) of heparinized red cells is given in Table 21.

Table 21. LEAD IN YOUNG AND OLD RBC

Subject	Hct%	Pb-B top 10% rbc (young cells)	Pb-B bottom 10% rbc (old cells)
A	49	105.2	89.2
B	41	76.5	60.7
C	37	82.0	67.2
D	30	78.0	78.7

There was a greater concentration of lead in the top layer of red cells in 3 of the 4 assays. The assay was done after reconstitution to the original hematocrit of the whole blood. Normally, the bottom layer of cells is denser with a lower hematocrit than the mean. Reconstitution to the mean or higher hematocrit should thus result in a higher concentration of lead unless there were differences in affinity for lead between the young cells of larger volume and larger surface area at the top and the shrunken older cells at the bottom.

Red Cell Membrane Na-K ATPase

A total of 65 blood samples were assayed for total and sodium-potassium adenosine triphosphatase (Na-K ATPase) activity of the rbc membrane. The subjects had a mean Pb-B of 22.7 ug/dl with a range of 4.9 - 93; all but 4 were below 40 ug/dl. The values in Table 22 are those of the 3 age groups plus values obtained on 2 lead workers. Total ATPase activity for the entire group was 1.57 ± 0.05 mM phosphate liberated/hour/mg protein (mean ± SE). Na-K ATPase or ouabain inhibited enzyme activity was 0.93 mM ± 0.30.

Within the total group there was no consistent correlation of total or Na-K ATPase activity with age, race, or hematocrit. No sex correlation had been noted, but the subjects were predominantly male. Serial assays of enzyme activity of individuals were relatively constant over

several months time. Although enzyme activity has been said to be increased in the young cell, the enzyme activity of cell membranes from 13 G-6-PD deficient boys, ages 9-14, did not differ significantly from that of the 7 urban black males of the same age, and these values are grouped with ages 10-18 in Table 22.

Table 22. BLOOD LEAD AND RED CELL MEMBRANE Na/K ATPase

	Pb-B µg/dl	Hct %	ATPase Total	ATPase Na/K
10-18 yr N = 49	18.5 ± 1.2	42.0 ± 0.5	1.69 ± 0.04	1.03 ± 0.04
2-5 yr N = 5	37.0 ± 1.8	36.5 ± 0.8	1.32 ± 0.06	0.73 ± 0.06
Adult N = 9	27.3 ± 2.6	45.1 ± 0.6	1.24 ± 0.06	0.70 ± 0.06
Lead workers	(1)92.0 (2)81.0	50.0 46.0	0.80 0.87	0.13 0.31
Total N = 65	22.7 ± 1.7	42.1 ± 0.3	1.57 ± 0.05	0.93 ± 0.04

All values given as mean ± SE. ATPase is measured as µMoles phosphate liberated per mg protein per hour.

In the 34 samples with Pb-B below 20 µg/dl, total ATPase activity was 1.67 ± 0.39, Na/K activity was 1.04 ± 0.25. In 27 samples with the Pb-B of 20-40 µg/dl, there was no significant depression of total ATPase activity, but Na/K ATPase (oubain inhibited activity) was significantly decreased to 0.89 ± 0.28 mM, $p < .05$ (Table 23).

Table 23. Na/K ATPase OF RED CELL MEMBRANE
BLOOD LEAD< 20 VS 20-40

	Blood Lead µg/dl		
	< 20 N = 34	20-40 N = 27	> 40 N = 4
Na/K ATPase	1.04* ± 0.04	0.89* ± 0.05	0.40 ± 0.10
Total ATPase	1.67 ± 0.07	1.53 ± 0.07	1.02 ± 0.09

*$p < .05$ by t for Na/K ATPase of Pb-B < 20 µg/dl vs Pb-B 20-40 µg/dl.

The correlation of Na/K ATPase activity with blood lead for the 5 populations is plotted in Figure 21. There is an inverse linear correlation, $r = -.36$, $p < .05$, with the regression coefficient of Na/K ATPase = 1.21 - 0.01 Pb-B. Elimination of the data from the 2 lead workers does not significantly alter the correlation or the regression equation.

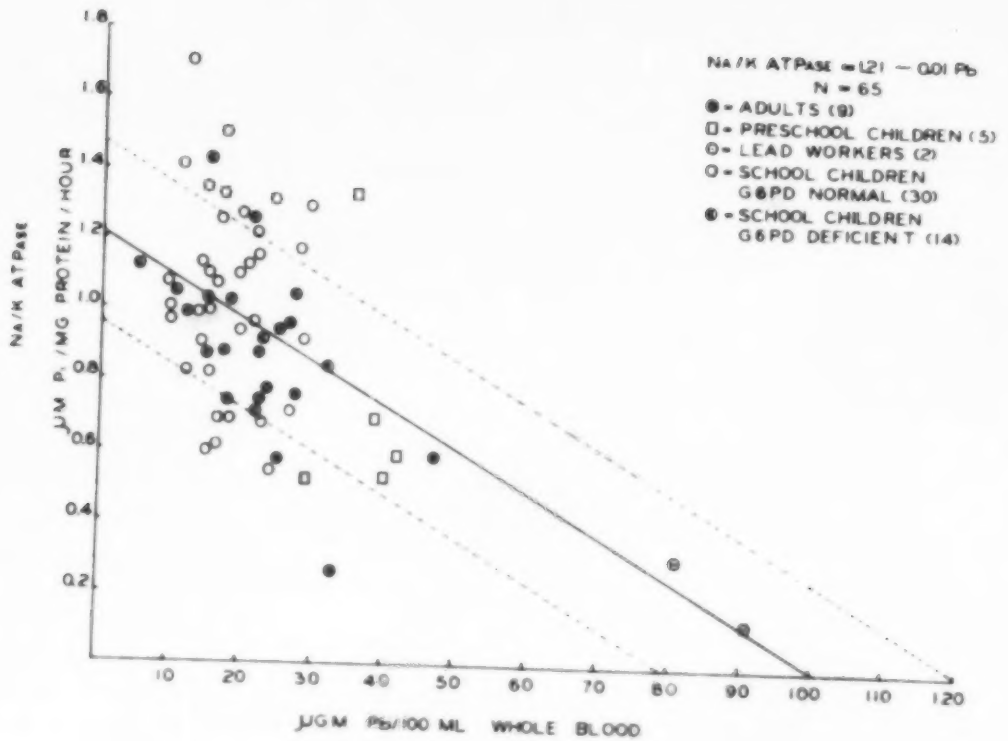

Figure 21. Correlation of blood lead with red cell membrane Na/K ATPase as assayed in 65 samples. The number of G-6-PD normal school children actually totals 35, not 30, as indicated on the label. The correlation of enzyme activity and blood lead, $r = -0.56$ is significant ($p < .05$).

SECTION VIII

DISCUSSION

A. ENVIRONMENTAL STUDIES

One of the most striking aspects of the study is the sharp drop in air lead in urban Omaha. By 1974, the lead in urban air was lower than the suburban values of 1970-1972 (Figure 10, p. 33). Although industrial air filters might be expected to remove relatively more of the large particles, there was less of a decrease in dustfall lead than in air lead (Figure 12, p. 36). This suggests that recirculation of the large particles of lead in street dirt and soil may be a factor in persistence of high dustfall lead even after a reduction in lead emission.

The fallout from air lead to dustfall and dirt shows a consistently better correlation with the urban-suburban differences in blood lead in all ages of children than does air lead itself[37,40,53]. In Omaha, lead in water and milk are insignificant. Lead in housedust, in this study, relates to the blood lead only in the preschool age group where mouthing behavior and long hours of playing on the floor might be expected to increase the ingestion of house dust to the levels of a significant load per unit of body weight.

Because of the drop in environmental lead we reached ahead to blood lead studies done in 1975 (ES 00939) and examined Pb-B as comparably assayed (macro method, spring season) in students at an urban junior high school in 1971, 1973, and 1975 (Table 2). There is a significant decrease in Pb-B from 1971 to 1975 and from 1973 to 1975 that parallels the decrease in air and dustfall leads:

Table 24. BLOOD LEAD, 1971-1975,
URBAN JUNIOR HIGH SCHOOL, AGES 12-14

May, 1971	March, 1973	March, 1975
25.1** ± 1.1 (32)	21.0* ± 2.4 (13)	16.1 ± 0.9 (17)

** $p < .001$, 1971 vs 1975
* $p < .05$, 1973 vs 1975

All blood leads done by macro method of Farrelly & Pybus on heparinized venous samples. Values are the mean ± SE, with the numbers of subjects given in parentheses. Data from this school was selected for analysis because of the consistency of methodology (macro assay) and the comparable season of sampling.
By *t* test there is a significant difference between 1971 and 1975 ($p < .001$) and 1973 and 1975 ($p < .05$).

Although the annual trend in Pb-B and its correlation with general environmental lead in air and dustfall can be seen, we were unable to relate the monthly and seasonal fluctuations in Pb-B to the monthly variations in air lead or dustfall lead. Soil lead was obtained in the standard two-inch core to avoid rapid fluctuations. To better evaluate a seasonal correlation of Pb-B with environmental lead, monthly samples of the top layer of soil or of street dirt would be more appropriate.

Despite the critical role of deteriorated housing as a cause of clinical lead poisoning in young children, the urban-suburban difference in housedust lead was much less obvious than the urban-suburban differences in dustfall and soil lead. High levels of dustfall and soil lead clearly related to industrial sources as shown by the comparison of the 2 urban schools. Dustfall lead at the urban high school averaged 9.4 $mg/M^2/30$ day collection. The urban grade school is in a comparable mixed residential-commercial area but is immediately adjacent to a battery plant - an industrial source that presumably accounts for a mean of dustfall lead of 22.7 $mg/M^2/30$ d. All soil lead samples adjacent

to this plant were below 2000 μg/gm, and far below the levels of soil lead in smelter studies such as that in El Paso[7].

Traffic as well as industry can be incriminated as a significant lead source even though the Omaha criterion of 3500 cars/lane/day is far, far below the traffic density in New Jersey as studied by Caprio et al[54]. Children living within 750 feet of an Omaha trafficway had a mean Pb-B of 27.3 vs 21.0 for those living at a greater distance and proximity to traffic related linearly to soil lead.

This environmental study in Omaha supports multiple surveys[2,7-12] conclusively documenting the higher lead load of the urban child. By school age, this increase in lead burden relates directly to the general environmental exposure to lead in proximity to traffic and industry.

B. Red Cell Metabolism and Iron Deficiency

This study of the effect of oral iron on the Pb-B was designed as a clinical trial of the experimental evidence that iron deficiency enhances the absorption and transport of lead[17-19]. The study can be criticized on the grounds that, 1) the subjects had only a low serum iron but no real anemia, 2) the daily dose of 200 mg ferrous sulfate is less than the therapeutic dose of 600 mg/day and was given only 5 days per week, 3) the response is obscured by the known seasonal fluctuations in both iron and lead. Despite these valid criticisms, the conditions of the study are those that might be accomplished in any broad scale test of the hypothesis. The fact that absolutely no decrease in Pb-B occurs on giving oral ferrous sulfate, 200 mg/d, 5 days a week, for 4 months supports previous studies showing no effect of iron on pica or on blood lead[55], and allows two other inferences. First, since Pb-B increases as the serum iron increases and as the hemoglobin increases, the correction of Pb-B for anemia remains physiologically valid. Second, the binding of lead by ferritin and transferrin may affect the kinetics of lead absorption in different ways at different concentrations and could explain the failure of physiologic levels of iron supplementation to interfere with lead absorption. Although saturation of ferritin and transferrin with iron may displace lead from a common transport system; iron supplementation short of saturation might facilitate lead transport by enzymatic induction or stimulation of another common carrier mechanism[56-58].

C. Red Cell Survival and Na/K ATPase

This is the first report of a linear inhibition of Na/K ATPase of the red cell membrane. As the blood lead increases within the normal range from 0 to 40 μg/100 ml[59-61], ALA dehydratase of the red cell is also linearly depressed; the health effects of this are unknown since ALA-D has no known metabolic activity in the mature red cells. Membrane Na/K ATPase, in contrast, is a critical determinant in cation permeability and red cell survival[29-30]. Almost 50 years ago, Aub documented that in vivo and in vitro exposure to lead causes a rapid loss of intercellular cation resulting in small, shrunken, hyperosmotic red cells[62], an effect similar to the inhibition of Na/K ATPase. In vitro exposure of rbc to lead evokes a rapid efflux of K^+ that occurs at rates so much faster than the maximum pumping capacity of the cell that it is considered a passive leak of potassium[25]. Since the potassium leak is reversed by EDTA, it is postulated that it represents the binding by lead of surface reactors on the membrane[34]. In vitro exposure to lead also causes a decrease in ability to pump sodium out of the red cell; unlike the potassium leak, this inhibition of the sodium pump is not reversed by treatment with EDTA and may represent lead binding to Na/K ATPase, or at least to a site other than the outer membrane.

Hasan and Hernberg were the first to show a decrease in Na/K ATPase, but not total ATPase, of industrial lead workers at Pb-B of 60-80 μg/dl [31,32]. Secchi and Alessio also found a lower Na/K ATPase in lead workers and subsequently reported decreases in membrane Na/K ATPase in urban men with increased occupational exposure to lead such as traffic policemen[33]. We modestly attribute our demonstration of a linear inhibition to improved methodology of the enzyme assay, since we were unable to demonstrate inhibition, even at high Pb-B, employing these previously reported methods of enzyme assay.

Since Na/K ATPase is a critical enzyme in integrity of the red cell membrane, we then investigated the evidence for decreased red cell survival in the same range of values. Red cell enzymes are well accepted indices of red cell age and of red cell survival. Although G-6-PD and 6-PGD are less sensitive indicators of red cell age than hexokinase or aldolase, demonstration of an increased value in children with a Pb-B above 20 μg/dl may be evidence of a decreased rbc survival in association with altered cation transport of the rbc membrane. Under varying experimental circumstances, lead may inhibit or enhance G-6-PD activity. Because G-6-PD activity did not change with Pb-B in G-6-PD deficient subjects, an enzyme increase relating to increased rbc turnover might be postulated. Since inhibition of GSH at low levels of Pb-B, similar to that recently

reported by Taniguchi[63], might be expected to depress G-6-PD activity, the intrepretation is proposed that increased G-6-PD represents accelerated turnover. Further studies of the effects of lead on Na/K ATPase and also on Ca^{++} ATPase of white cells and platelets would seem indicated.

SECTION IX

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SECTION X

GLOSSARY

ATPase - adenosine triphosphatase.

2, 3 DPG - 2, 3 diphosphoglycerate.

Fe Saturation - $\frac{\text{Serum iron}}{\text{Total iron binding capacity}} \times 100\%$.

G-6-PD - Glucose-6-phosphate dehydrogenase.

GOT - Glutamic oxalacetic transaminase.

6-PGD - 6-phosphogluconic dehydrogenase.

GSH - Reduced glutathione.

Hct - Hematocrit.

Hgb - Hemoglobin.

MCV - Mean corpuscular volume of red cells.

Na/K ATPase - sodium-potassium activated or oubain inhibited adenosine triphosphatase.

Pb-B - Blood lead in µg/dl.

Rbc - Red blood cell.

Serum Fe - serum iron.

TIBC - Total iron binding capacity of serum or serum transferrin.